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Synthesis of Optically Pure Norcantharidin Analogue NCA-01, a Highly Selective Protein Phosphatase 2B Inhibitor, and its Derivatives

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Abstract: An efficient synthetic route to optically pure norcantharidin analogue NCA-01, a highly selective inhibitor of protein phosphatase 2B (PP2B; calcineurin), has been developed. The absolute stereochemistry of the enantiomers was determined by X-ray crystallographic analysis. Optically pure NCA derivatives that had various substituents at the C1 position were synthesized in a similar manner. The PP2B-inhibitory activities of NCA-01 and its derivatives were independent of the enantiomeric form. NCA-01 dimethyl ester potently inhibited IL-2 production in Jurkat cells.

Keywords: biological activity · calcineurin · inhibitors · norcantharidin · protein phosphatase

Introduction

Protein phosphatases are key enzymes in signal-transduction pathways, by regulating entry into the cell cycle, mitosis, and cell death. They can be categorized into three groups: protein serine/threonine-specific, tyrosine-specific, and dual-specific phosphatases. Based on their biological properties, substrate specificity, and sensitivity to specific inhibitors, serine/threonine phosphatases have been further classified into several subtypes, and, among them, protein phosphatase 1 (PP1), protein phosphatase 2A (PP2A), and protein phosphatase 2B (PP2B) have been well-studied.[1,2]

PP2B (also called calcineurin) is a calcium- and calmodulin-dependent enzyme that is composed of calcineurin A (catalytic subunit; 60 kDa), which shares high sequence homology with PP1, PP2A, and calcineurin B (calcium/calmodulin-binding subunit; 15 kDa). PP2B is distributed widely in the brain and lymphocytes and has established roles in T-cell activation, neuronal cell death, vesicular trafficking, and learning and memory.[3] Immunosuppressant drugs cyclosporine A (CsA) and FK506 inhibit PP2B and block activation of transcription factor NF-AT in T-lymphocytes.[4] These drugs bind to immunophilins (cyclophilin and FKBP, respectively) and the resulting complexes inhibit the phosphatase activity of PP2B. Remarkably, these immunosuppressants cannot directly inhibit PP2B, and the formation of the immunophilin complex is essential. Because immunophilins are involved in many cellular processes, such as the regulation of calcium ion channels, protein folding, and cell death, FK506 and CsA may cause a variety of side-effects. Thus, it is of interest to find direct and selective inhibitors of PP2B that do not bind to immunophilins, both as biological tools for studies of PP2B and as candidate therapeutic agents.[5–11]

There are several reports on natural and synthetic products that inhibit PP2B without binding immunophilin, but these compounds have potential problems. Polypeptide PP2B inhibitors are potent and specific, but may have limited cell-permeability and short lifetimes.[5] Although pyrethroid insecticides, such as fenvalerate, cypermethrin, and permethrin, have been reported to be potent and specific PP2B inhibitors,[6a] their effectiveness has been disputed by other groups.[6b,c] Dibefurin,[7] PD144795,[8] and tyrphostin derivatives[9] have also been reported to inhibit PP2B, but their selectivity remains unknown. Gossypol is a potent inhibitor of PP2B ($IC_{50}=16\ \mu M$), but although it shows negligible inhibition of other protein phosphatases,[10a] it inhibits other enzymes, such as GgIP3K-A ($IC_{50}=58$ nM).[10c] Recently, aminoalkyl-substituted diarylheterocycles have been reported to inhibit PP2B without inhibiting other protein phosphatases and immunophilins and to be effective on the cell level,[11] whereas there is still a need for direct and selective PP2B inhibitors.

Cantharidin was isolated from the dried body of the Chinese blister beetle (*Mylabris phalerata* or *M. cichorii*) in

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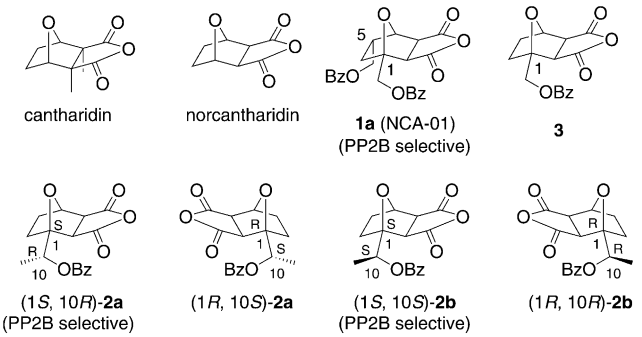
1810, and it has been used as a medicinal agent for over 2000 years. It is a moderately potent inhibitor of PP1 and PP2A, but it inhibits PP2B only very weakly.[11] Although numerous analogues of cantharidin have been reported as potent and selective inhibitors of PP1 and PP2A in recent years,[12] only a few were evaluated as PP2B inhibitors.[13]

We have previously reported that the racemic 1,5-disubstituted norcantharidin derivative **1a** (NCA-01) is a highly selective catalytic site-directed PP2B inhibitor that shows negligible inhibition of PP1 and PP2A (Scheme 1).[14] It is important to evaluate the activity of each enantiomer to obtain a better understanding of the interaction between the inhibitor and the enzyme. However, our efforts to separate the enantiomers of compound **1a** by chiral-phase HPLC were unsuccessful, owing to the lability of the acid anhydride moiety. Therefore, we prepared two optically active mono-substituted norcantharidin analogues (**2a** and **2b**) starting from optically pure 1-(2-furyl)ethanol, and we evaluated all four stereoisomers.[15] Interestingly, isomers (1*S*,10*R*)-**2a** and (1*S*,10*S*)-**2b** showed high selectivity for PP2B and their inhibition of PP1 and PP2A was negligible. On the other hand, isomers (1*R*,10*S*)-**2a** and (1*R*,10*R*)-**2b** showed significant inhibition of all three PPs. These results indicated that the absolute stereochemistry at the C1 position was important for the selectivity. Furthermore, isomer (1*S*,10*S*)-**2b** showed more-potent PP2B-inhibitory activity than diastereomer (1*S*,10*R*)-**2a** and (1*S*)-benzoyloxymethyl derivative **3**, thus suggesting the importance of SAR study for the C1 substituent. These facts encouraged us to synthesize optically pure compound **1a** and its derivatives with various C1 substituents. Herein, we report a versatile synthetic route to optically pure compound **1a** and its derivatives **1b**–**1j**. The inhibitory activity of these compounds towards PP1, PP2 A, and PP2B was also evaluated.

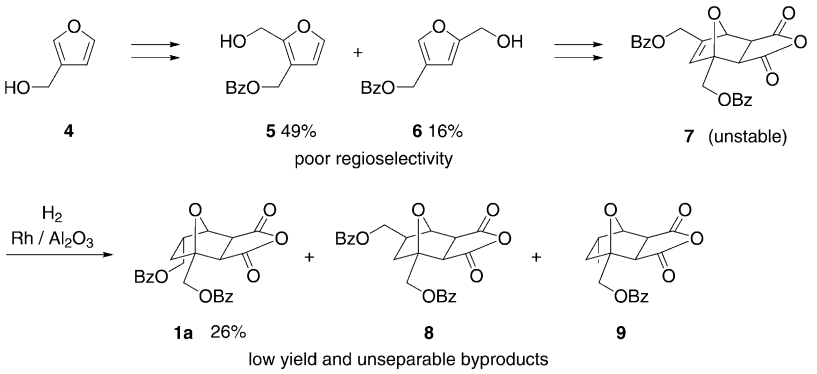


Scheme 1. Structures of cantharidin, norcantharidin, and cantharidin analogues synthesized by ourselves.

Results and Discussion

Synthetic Route to Optically Pure NCA-01 and its Derivatives

In addition to the difficulty of separation of the enantiomers, our original synthetic route (Scheme 2) had several problems. The 2,4-dibenzoyloxymethylfuran (**6**) was synthesized from 3-furanmethanol by benzoylation, Vilsmeier reaction, and reduction steps. However, the selectivity of the Vilsmeier reaction was poor and the desired regioisomer was only a minor product (16% yield over 3 steps). The key Diels–Alder reaction and the subsequent hydrogenation reaction were also troublesome. The yield of the desired product (**1a**) was low, owing to the formation of stereoisomer **8** and hydrogenolysis product **9** in addition to the retro-Diels–Alder product.



Scheme 2. Problems in the original synthetic route to compound **1a**.

To overcome these problems, we planned a versatile synthetic route to the optically active 1,5-disubstituted norcantharidin derivatives (**1**), as shown in Scheme 3. To allow for the introduction of a variety of acyl groups at the C1 position after separation of the enantiomers, we selected the

Abstract in Japanese:

プロテインホスファターゼ２Ｂ阻害活性を有する 1, 5-二置換ノルカンタリジン誘導体（NCA 誘導体）を光学活性体として合成する方法を開発し、さまざまな１位置換基をもつ NCA 誘導体の両光学活性体の合成を行った。合成した光学活性体の絶対立体配置はＸ線結晶構造解析法により決定した。合成した光学活性 NCA 誘導体についてプロテインホスファターゼ２Ｂ阻害活性試験を行ったところ、鏡像異性体間の活性の差がほとんど見られないことが明らかとなった。さらに、化学的に安定で細胞内酵素により加水分解をうけると期待されるジエステル誘導体 NCA-01-ME が Jurkat 細胞の IL-2 産生を抑制することを見出した。

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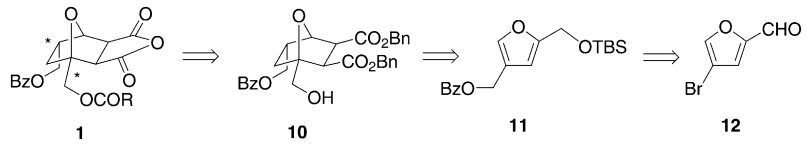
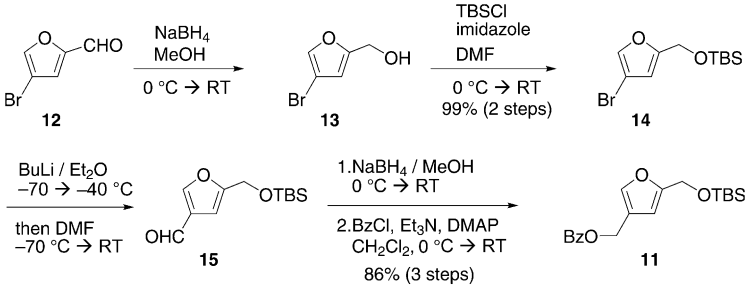


Scheme 3. Synthetic route to optically active NCA derivatives.

stable diester **10** as a key intermediate. We expected that 4-benzoyloxymethyl-2-*tert*-butyldimethylsilyloxymethylfuran (**11**) would be obtainable from the commercially available 3-bromofurfuryl aldehyde (**12**).

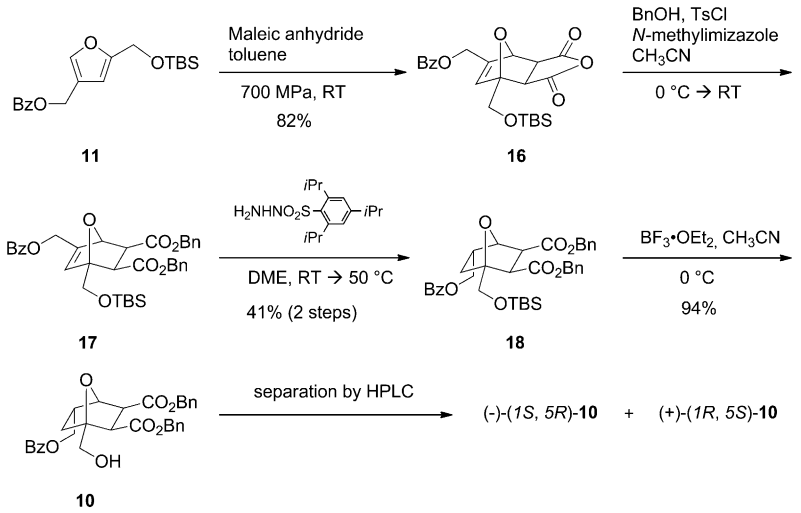
First, 3-bromofurfuryl aldehyde (**12**) was converted into alcohol **13**, which was protected with a *tert*-butyldimethylsilyl group (TBS) to give compound **14** (Scheme 4). Lithiation



Scheme 4. Synthesis of 2,4-disubstituted furan derivative **11**.

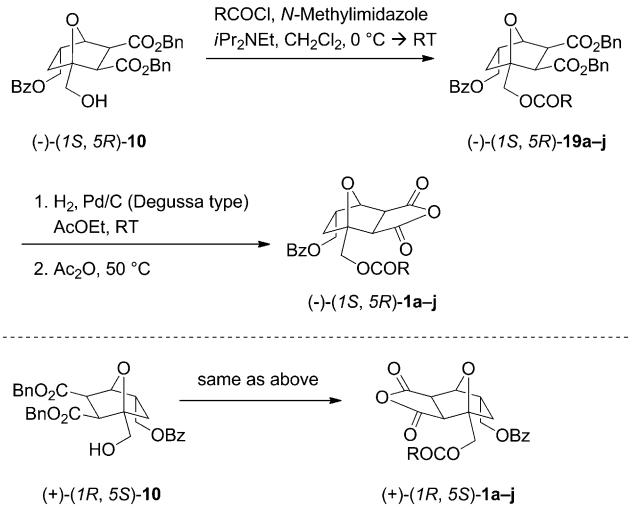
of compound **14**, followed by the addition of *N*,*N*-dimethylformamide (DMF), afforded aldehyde **15**.[16] Reduction of the formyl group and subsequent benzoylation of the alcohol furnished the desired 2,4-disubstituted furan derivative (**11**).

The Diels–Alder reaction of compound **11** with maleic anhydride in toluene at 50 °C for 4 days produced cycloadduct **16** in 72 % yield (Scheme 5). We also examined the reaction of compound **11** at high pressure. The Diels–Alder reaction of compound **11** with maleic anhydride in CH_2Cl_2 at 700 MPa was complete within 24 hours, thus affording compound **16** in 82 % yield. However, for the large-scale reaction, we preferred to use the former conditions. The key hydrogenation reaction of cycloadduct **16** was examined by using various metal catalysts, such as Rh/Al_2O_3, Rh/C, PtO_2, and $[Ir(cod)(Py)(PCy_3)]^+PF_6^-$ (cod = 1,5-cyclooctadiene). Unfortunately, the yield of the desired product was not improved. We also examined the diimide reduction of the double bond, but the acid anhydride group was not compatible with the hydrazine derivatives. Therefore, we investigated the conversion of compound **16** into the corresponding benzyl ester (**17**). All of the reactions of compound **16** with benzyl alcohol in the presence of various coupling reagents, such as DCC (DCC = *N*,*N*′-dicyclohexylcarbodiimide), DCC-HOBt (HOBt = 1-hydroxy-1*H*-benzotriazole), EDC-HOOBt (EDC = 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide, HOOBt = 3-hydroxy-3,4-dihydro-4-oxo-1,2,3-benzotriazine), and 2-chloropyridinium iodide, in the absence or presence of base, such as DMAP, *i*Pr_2NEt, and Et_3N, under ambient conditions, were unsuccessful. Significant epimerization at the C2 or C3 positions was observed, in addition to the retro-Diels–Alder reaction. Finally, we were pleased to find that the reaction of compound **16** with benzyl alcohol in the presence of TsCl (Ts = 4-toluenesulfonyl) and *N*-methylimidazole gave the desired dibenzyl ester (**17**) without epimerization of the C2 and C3 positions.[17] Diimide reduction of compound **17** by using 2,4,6-triisopropylbenzenesulfonohydrazide proceeded smoothly to afford the desired product (**18**) as a single diastereoisomer. The key intermediate (**10**) was obtained by deprotection of the TBS group of compound **18** with boron trifluoride diethyl etherate. As expected, the separation of its enantiomers was achieved by preparative HPLC by using a chiral stationary phase (CHIRALPAK® IA), and optically pure compounds (−)-**10** and (+)-**10** were obtained.



Scheme 5. Synthesis of common chiral intermediate **10**.

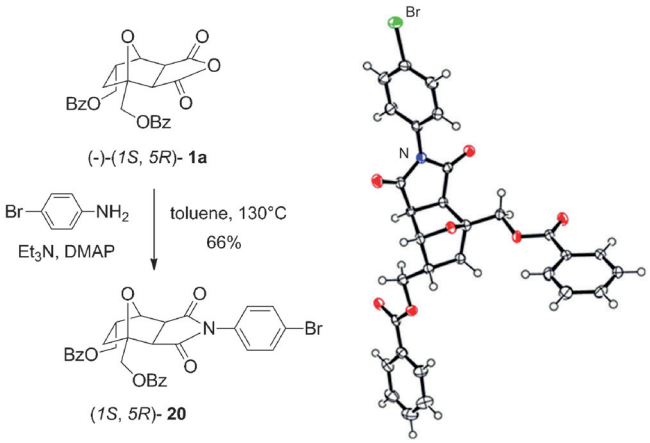
First, compound (−)-**10** was transformed into (−)-NCA-01 (**1a**) by a three-step sequence. Benzoylation of the hydroxy group with benzoyl chloride afforded compound (−)-**19a**. Finally, acid anhydride (−)-**1a** was obtained by hydrogenolysis of the benzyl ester of compound (−)-**19a**, followed by treatment with acetic anhydride. Using the same synthetic route, compound (+)-**10** was



Scheme 6. Synthesis of optically active NCA derivatives.

converted into (+)-**1a** (Scheme 6). Furthermore, both enantiomers of NCA derivatives **1b**–**1j**, which had various acyl groups at the C1 position, were also synthesized in a similar manner.

The absolute stereochemistry of compounds (−)-**10** and (−)-**1a** were determined to be (*1S*,*5R*) by X-ray crystallography of compound **20**, which was derived from compound (−)-**1a** by an imide-formation reaction with 4-bromoaniline under the influence of triethylamine and 4-dimethylaminopyridine (DMAP; Scheme 7). The absolute stereochemistry of all other derivatives was determined by correlation to compound **10**.



Scheme 7. Preparation of (1*S*,5*R*)-**20** and an ORTEP of its crystal structure.[21]

Effect of the Absolute Stereochemistry on PP2B-Inhibitory Activity

Initially, the PP2B-inhibitory activity of enantiomers of NCA-01 (**1a**) were examined in terms of their ability to inhibit the dephosphorylation of an exogenous substrate (R-II phosphopeptide) by human recombinant PP2B.[14] Unexpectedly, the IC_{50} values of (*1S*,*5R*)-**1a** and (*1R*,*5S*)-**1a** were equal (5.2 μM).

To understand this result, we carried out the molecular docking of both enantiomers of compound **1a** with the PP2B active site based on the reported crystal structure (PDB ID: 1TCO)[18] using DS Modeling 1.2 (Accelrys). Figure 1 shows the binding modes of the dicarboxylate form

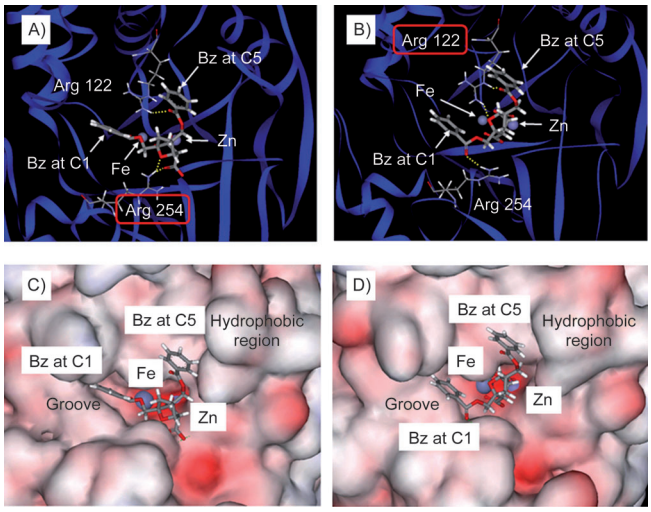


Figure 1. A, C) Binding model of (1*S*,5*R*)-**1a** with the PP2B active site. B, D) Binding model of (1*R*,5*S*)-**1a** with the PP2B active site.

of isomers (1*S*,5*R*)-**1a** (A and C) and (1*R*,5*S*)-**1a** (B and D) to PP2B.

In the X-ray structure of the PP2B–phosphate-anion complex, the phosphate oxygen atoms coordinated to the metal ions in the catalytic site (Zn, Fe). The conserved arginine residues, Arg122 and Arg254, may play a role in substrate-binding by forming hydrogen bonds with the oxygen atom of the phosphate ester.[3b]

In our binding models of isomers (1*S*,5*R*)-**1a** and (1*R*,5*S*)-**1a**, the carboxylate moieties were situated at positions in which ionic interactions between the oxygen atoms of the carboxylate groups and the metal ions were possible. However, the orientation of the bridging ether oxygen was different for the two enantiomers. A hydrogen bond between the bridging ether oxygen of the (1*S*,5*R*)-isomer and the Arg254 residue was observed in binding model A, whereas the (1*R*,5*S*)-isomer formed a hydrogen bond with Arg122 in binding model B. As a result, the C1 substituent of both enantiomers of compound **1a** fit well into the deep groove of PP2B, and the C5 substituent interacted with the hydrophilic region, which was characteristic of PP2B, but not of PP1 or PP2A.

Recently, the crystal structure of the complex of protein phosphatase 5 (PP5c) with norcantharidin was reported.[19] As predicted, norcantharidin was bound as its dicarboxylate form. Interestingly, multiple binding modes of the norcantharidin dicarboxylate core structure were observed, thereby suggesting flexible interactions between the core dicarboxy-

late structure and the conserved catalytic-site structure of the Ser/Thr protein phosphatases, which was composed of two metal ions and basic amino acid residues. This result seemed to be consistent with the finding that both enantiomers of compound **1a** bound PP2B with similar affinity.

Effect of the C1 Substituent on the PP2B-Inhibition Activity

To examine the effect of the C1 substituent on the potency and selectivity of PP2B inhibition, we evaluated various NCA derivatives, **1b**–**1j**. The percentage inhibition of both enantiomers of these NCA derivatives are summarized in Table 1. NCA-01 showed nearly 70% inhibition of PP2B activity. The introduction of a methyl group at the *ortho* (**1b**), *meta* (**1c**), or *para* (**1d**) position on the phenyl ring did not greatly affect the PP2B-inhibition activity. The replacement of the phenyl ring with a cyclohexyl (**1e**) or cyclopentyl ring (**1f**) that had similar hydrophobicity was also well-tolerated, but its replacement with a cyclopropyl group (**1g**) reduced the activity. Although no significant difference was generally observed between the enantiomers, isomer (1*R*,5*S*)-**1g** was less potent than the (1*S*,5*R*) isomer. NCA derivatives that had branched alkyl substituents, such as *t*Bu (**1h**) and *i*Pr (**1i**), showed effective PP2B inhibition, whereas the derivative that contained a small acetyl group (**1j**) showed a significant loss of potency. These results suggested that the hydrophobicity and size of the substituents at the C1 ester group were important for the PP2B-inhibition activity. Furthermore, almost all of the NCA derivatives had little or no inhibitory effect on PP1 and PP2A, even at 100 μM concentration, regardless of the size of the C1 substituent and the absolute stereochemistry. We have previously reported that the introduction of a hydrophobic substituent at the C5 position of norcantharidin led to an increase in the PP2B-inhibition activity[13a] and binding to PP1 and PP2A was blocked in the presence of a C1 benzoyloxymethyl group.[14] The SAR results (Table 1) indicated that, in the presence of the C5 benzoyloxymethyl group, a small substituent, such as an acetyl group, at the C1 position was sufficient to block the binding to PP1 and PP2A. However, the additional interaction of the C1 acyl group with the hydrophobic amino acid residues in the groove may be required for the high binding affinity to PP2B.

Table 1. Inhibitory activity of optically pure NCA derivatives towards PP2B, PP1, and PP2A.

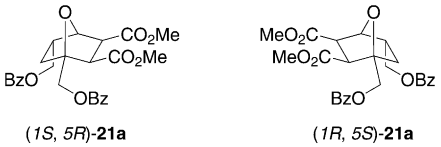
Compound	R	PP2B Inhibition [%] (10 μM)		PP1 Inhibition [%] (100 μM)		PP2A Inhibition [%] (100 μM)	
		(1*S*,5*R*)	(1*R*,5*S*)	(1*S*,5*R*)	(1*R*,5*S*)	(1*S*,5*R*)	(1*R*,5*S*)
1a	C_6H_5	71±3.7	65±1.5	ni	ni	ni	ni
1b	2-CH_3-C_6H_4	72±7.2	61±3.7	ni	ni	ni	ni
1c	3-CH_3-C_6H_4	75±6.3	58±4.6	ni	ni	ni	ni
1d	4-CH_3-C_6H_4	63±8.2	60±2.7	ni	ni	ni	ni
1e	*c*-C_6H_{11}	71±4.1	61±5.4	ni	ni	ni	ni
1f	*c*-C_5H_9	68±1.1	54±6.2	ni	ni	ni	ni
1g	*c*-C_3H_5	48±9.0	25±6.8	ni	ni	ni	ni
1h	*tert*-C_4H_9	62±1.3	50±4.4	ni	ni	ni	ni
1i	*i*-C_3H_7	62±6.5	45±7.1	ni	ni	ni	ni
1j	CH_3	17±10	15±14	ni	ni	ni	ni

ni = no inhibition was observed.

Inhibition of IL-2 Production in Jurkat Cells by Optically Active NCA-ME Derivatives

It is known that the stimulation of Jurkat cells with mitogen induces the production of IL-2 through the activation of PP2B and NF-AT. Because PP2B inhibitors, such as cyclosporin A, are known to suppress IL-2 production, we investigated the effect of NCA-01 at the cell level. However, the inhibition of IL-2 production in Jurkat cells by NCA-01 was very weak. We speculated that the acid anhydride group in NCA-01 would be easily hydrolyzed in aqueous solution to give the dicarboxylic acid derivative, which could strongly bind PP2B, but was expected to have poor cell permeability. Therefore, we prepared the more-chemically stable dimethyl ester derivative of NCA-01, NCA-01-ME (**21a**), as a pro-drug of NCA-01 dicarboxylate (Scheme 8). Although neither of the enantiomers of compound **21a** inhibited PP2B, we expected that the methyl ester moieties would be hydrolyzed to dicarboxylate by endogenous esterases in cells.

Using a reported procedure,[20] we quantified IL-2 production in the supernatant of Jurkat T lymphocytes that were stimulated with PHA (phytohemagglutinin) in the presence or absence of optically active NCA-01-ME derivatives (30 μM). The cytotoxicity of these compounds to Jurkat cells was also measured by an AlamarBlue assay.



(*1S, 5R*)-**21a** (*1R, 5S*)-**21a**

Scheme 8. Structures of NCA-01-ME derivatives (1*S*,5*R*)-**21a** and (1*R*,5*S*)-**21a**.

As expected, the addition of 30 μM of NCA-ME derivative (1*S*,5*R*)- or (1*R*,5*S*)-**21a** suppressed the production of PHA-stimulated IL-2 without affecting the cell viability. These results indicated that the inhibitory effect of NCA-ME derivatives on IL-2 production did not involve cytotoxicity (Table 2).

Table 2. Inhibitory effects of NCA-ME derivatives on IL-2 production in Jurkat cells.

Compound	Inhibition [%] (μM)	Cell viability [%] (μM)
DMSO	0	100±4.6
cyclosporine A	100±8.7 (1)	94±0.30 (1)
(1*S*,5*R*)-**21a**	84±17 (30)	104±6.3 (30)
(1*R*,5*S*)-**21a**	73±6.0 (30)	107±1.4 (30)

Conclusions

An improved synthetic route to optically active NCA derivatives was developed and the absolute stereochemistry of the products was determined by X-ray crystallographic analysis. The PP2B-inhibitory activity of the enantiomers of NCA-01 was independent of the enantiomeric form. Molecular modeling suggested that the two enantiomers bound to PP2B in different modes. Furthermore, we synthesized a series of 5-benzoyloxymethylnorcantharidin derivatives with various substituents at the C1 position and evaluated their inhibitory activities toward PP1, PP2A, and PP2B. The NCA derivatives that had hydrophobic substituents at the C1 position inhibited PP2B but showed negligible inhibition of PP1 and PP2A, whilst the dimethyl ester NCA-01-ME derivative (**21a**) significantly inhibited IL-2 production in Jurkat cells. These compounds are the first step in the development of PP2B inhibitors that are effective on the cellular level and are expected to be useful tools for future studies on the function of PP2B.

Experimental Section

General Methods

^{1}H NMR Spectra were recorded on JEOL JNM-AL-300 and AL-400 spectrometers, which operated at 300 and 400 MHz, respectively. ^{13}C NMR Spectra were recorded on a AL-400 spectrometer, which operated at 100 MHz. ^{1}H and ^{13}C NMR shifts are reported downfield from $CHCl_3$ ($\delta = 7.26$ and 77.0 ppm). MS (FAB) was performed on a JEOL JMA-HM110 mass spectrometer by using *m*-nitrobenzyl alcohol (*m*NBA). Column chromatography was performed with silica gel 60 (40–50 μm and 40–100 μm) that was purchased from Kanto Chemical Co. Preparative HPLC was performed on a JMM LC-6 A. Analytical HPLC was done on a Waters HPLC system or on a TOSOH HPLC system. Preparative TLC was performed with Silica gel 60 F_{254} (layer: 1 mm and 0.5 mm) that was purchased from Merck Ltd. All reactions were monitored by TLC on 0.25 mm E. Merck silica gel plates 60 F_{254} by using UV light or 10% ethanolic phosphomolybdic acid solution and heat as developing agents.

*4-Bromo-2-tert-butyldimethylsilyloxymethylfuran (**14**)[16]*

To a solution of 4-bromo-2-furaldehyde (**12**, 3.0 g, 17.2 mmol) in MeOH (90 mL) was added $NaBH_4$ (980 mg, 25.8 mmol) at 0 °C. The reaction mixture was stirred for 10 h at RT, then a saturated aqueous solution of NH_4Cl was added. The aqueous layers were extracted with EtOAc, and the combined organic layers were dried over anhydrous $MgSO_4$ and concentrated under reduced pressure. The crude product was dissolved in DMF (18 mL) under a nitrogen atmosphere, and imidazole (3.6 g, 52.6 mmol) and TBDMSCl (3.2 g, 21 mmol) were added at 0 °C. The reaction mixture was stirred for 2 h at RT, then brine was added. The aqueous layer was extracted with EtOAc, and the combined organic layer was dried over anhydrous $MgSO_4$ and concentrated under reduced pressure. The crude product was purified by column chromatography on silica gel (*n*-hexane/EtOAc, 4:1) to give compound **14** as a colorless oil (4.8 g, 99% over 2 steps). ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 0.09$ (s, 6H), 0.91 (s, 9H), 4.60 (s, 2H), 6.29 (d, $J = 0.7$ Hz, 1H), 7.36 ppm (d, $J = 0.7$ Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = -5.1$ (2C), 18.5, 25.9 (3C), 58.1, 99.9, 110.4, 140.0, 155.2 ppm; MS (FAB, positive-ion mode, *m*-nitrobenzyl alcohol): calcd for $C_{11}H_{20}BrO_2Si$: 291 $[M+H]^+$; found: 291.

*4-Benzoyloxymethyl-2-tert-butyldimethylsiloxymethylfuran (**11**)*

After azeotropic evaporation of compound **14** (1.85 g, 6.34 mmol) with toluene, to a solution of **14** in dry Et_2O (11 mL) was slowly added a solution of *n*BuLi (4.40 mL, 6.98 mmol, 1.59 M in *n*-hexane) at −70 °C under a nitrogen atmosphere. The reaction mixture was stirred for 1 h at −40 °C, then DMF (540 μL, 6.98 mmol) was added at −70 °C. Stirring was continued for 1 h at −40 °C and for 2 h at RT, then a saturated aqueous solution of NH_4Cl was added. The aqueous layer was extracted with EtOAc and the combined organic layers were dried over anhydrous $MgSO_4$ and concentrated under reduced pressure. The crude product (**15**)[16] was dissolved in MeOH (30 mL), and $NaBH_4$ (350 mg, 9.40 mmol) was added at 0 °C. The reaction mixture was stirred for 12 h at RT, then a saturated aqueous solution of NH_4Cl was added. The aqueous layer was extracted with EtOAc and the combined organic layers were dried over anhydrous $MgSO_4$ and concentrated under reduced pressure. The crude product, Et_3N (1.8 mL, 13 mmol), and DMAP (79 mg, 0.65 mmol) were dissolved in dry CH_2Cl_2 (15 mL), and benzoyl chloride (900 μL, 7.2 mmol) was added to the solution at 0 °C. The reaction mixture was stirred at RT for 3 h. The reaction was quenched with saturated sodium hydrogen carbonate, and the mixture was extracted with EtOAc. The organic layer was washed with water and brine, dried over anhydrous $MgSO_4$, and concentrated in vacuo. The crude product was purified by column chromatography on silica gel (*n*-hexane/EtOAc, 8:1) to give compound **11** as a colorless oil (1.90 g, 86% over 3 steps). ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 0.09$ (s, 6H), 0.90 (s, 9H), 4.61 (s, 2H), 5.19 (s, 2H), 6.35 (s, 1H), 7.45 (dd, $J = 7.1$ and 7.8 Hz, 2H), 7.48 (s, 1H), 7.55 (t, $J = 7.8$ Hz, 1H), 8.04 ppm (d, $J = 7.1$ Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = -5.1$ (2C), 18.5, 26.0 (3C), 58.2, 58.4, 108.1, 121.0, 128.2 (2C), 129.5 (2C), 130.0, 132.9, 140.9, 155.1, 166.3 ppm; MS (FAB, positive-ion mode, *m*-nitro benzyl alcohol): calcd for $C_{19}H_{27}O_4Si$: 347 $[M+H]^+$; found: 347.

(1S,2R*,3S*,4R*)-5-Benzoyloxymethyl-1-tert-butyldimethylsiloxymethyl-7-oxabicyclo[2.2.1]hept-5-ene-2,3-dicarboxylic anhydride (**16**)*

Ambient conditions: A mixture of compound **11** (2.6 g, 7.5 mmol) and maleic anhydride (2.2 g, 23 mmol) in toluene (0.5 mL) was stirred at 50 °C for 4 days. The reaction mixture was cooled to RT and the crude product was purified by column chromatography on silica gel (*n*-hexane/EtOAc, 4:1) to give compound **16** as a white solid (2.4 g, 72%).

High-pressure conditions: A mixture of compound **11** (350 mg, 1.01 mmol) and maleic anhydride (120 mg, 1.21 mmol) was placed in a Teflon reaction vessel with CH_2Cl_2 (1.0 mL) and pressurized to 700 MPa at RT for 5 h. After release of the pressure, the reaction mixture was concentrated in vacuo, and purified by column chromatography on silica gel (*n*-hexane/EtOAc, 4:1) to give compound **16** as a white solid (370 mg, 82%). M.p.: 85–86 °C; ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 0.10$ (s, 3H), 0.13 (s, 3H), 0.91 (s, 9H), 3.25 (d, $J = 6.6$ Hz, 1H), 3.46 (d, $J = 6.6$ Hz, 1H), 4.07 (d, $J = 11.0$ Hz, 1H), 4.25 (d, $J = 11.0$ Hz, 1H), 5.02 (d, $J = 1.5$ Hz, 2H), 5.4 (s, 1H), 6.47 (t, $J = 1.5$ Hz, 1H), 7.48 (dd, $J = 7.8$ and 7.8 Hz, 2H), 7.61 (dt, $J = 1.4$ and 7.8 Hz, 1H), 8.04 ppm (dd, $J = 1.4$ and 7.8 Hz, 2H); MS (MALDI-TOF, positive-ion mode, α-cyano-4-hydroxycinnamic acid): calcd for $C_{23}H_{28}NaO_7Si$ 467.15 $[M+Na]^+$; found: 467.23.

(1S,2R*,3S*,4R*,5R*)-5-Benzoyloxymethyl-1-tert-butyldimethylsiloxymethyl-7-oxa-bicyclo[2.2.1]heptane-2,3-dicarboxylic acid dibenzyl ester (**18**)*

To a solution of compound **16** (2.81 g, 6.30 mmol) and *N*-methylimidazole (3.0 mL, 37.8 mmol) in CH_3CN (12 mL) was added TsCl (2.65 g, 13.9 mmol) at 0 °C under a nitrogen atmosphere. The reaction mixture was stirred for 30 min and a solution of benzyl alcohol (2.87 mL, 27.7 mmol) in CH_3CN (12 mL) was added at 0 °C. The reaction mixture was stirred for 24 h at RT and then water was added. The aqueous layer was extracted with EtOAc and the combined organic layers were washed with water and brine, dried over anhydrous $MgSO_4$, and concentrated in vacuo.

To a solution of crude compound **17** in dry 1,2-dimethoxyethane (DME, 50 mL) was added 2,4,6-triisopropyl benzenesulfonyl hydrazide (3.3 g, 11 mmol) at RT and the reaction mixture was stirred at 50 °C for 5 h. The

reaction was quenched with saturated sodium hydrogen carbonate, and the mixture was extracted with EtOAc. The organic layer was washed with water and brine, dried over anhydrous $MgSO_4$, and concentrated in vacuo. The crude product was purified by column chromatography on silica gel (*n*-hexane/EtOAc, 6:1) to give compound **18** as a white solid (1.66 g, 41% over 2 steps). ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = -0.04$ (s, 3H), 0.00 (s, 3H), 0.83 (s, 9H), 1.26 (dd, $J = 6.3$ and 12.3 Hz, 1H), 2.16 (dd, $J = 12.0$ and 12.3 Hz, 1H), 2.74 (ddddd, $J = 4.9$, 6.0, 6.3, 10.0, and 12.0 Hz, 1H), 3.25 (d, $J = 9.8$ Hz, 1H), 3.44 (d, $J = 9.8$ Hz, 1H), 3.79 (d, $J = 10.4$ Hz, 1H), 3.92 (d, $J = 10.4$ Hz, 1H), 4.16 (dd, $J = 10.0$ and 11.3 Hz, 1H), 4.52 (dd, $J = 6.0$ and 11.3 Hz, 1H), 4.82 (d, $J = 12.1$ Hz, 1H), 4.88 (d, $J = 12.3$ Hz, 1H), 4.90 (d, $J = 12.1$ Hz, 1H), 5.06 (d, $J = 12.3$ Hz, 1H), 5.16 (d, $J = 4.9$ Hz, 1H), 7.22–7.31 (m, 10H), 7.42 (dd, $J = 7.8$ and 7.8 Hz, 2H), 7.56 (dt, $J = 1.4$ and 7.8 Hz, 1H), 7.98 ppm (dd, $J = 1.4$ and 7.8 Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = -5.6$, -5.3, 18.2, 25.7 (3C), 36.1, 40.5, 46.8, 54.2, 62.7, 64.6, 66.5, 66.8, 79.2, 89.3, 128.19 (2C), 128.21 (2C), 128.30 (2C), 128.44 (4C), 128.5 (2C), 129.58 (2C), 129.62 (2C), 133.2, 135.4, 166.2, 170.0, 170.4 ppm; MS (FAB, positive-ion mode, *m*-nitrobenzyl alcohol) calcd for $C_{37}H_{45}O_8Si$: 645 $[M+H]^+$; found: 645.

(1S,2R*,3S*,4R*,5R*)-5-Benzoyloxymethyl-1-hydroxymethyl-7-oxa-bicyclo[2.2.1]heptane-2,3-dicarboxylic acid dibenzyl ester (**10**)*

To a solution of compound **18** (1.66 g, 2.57 mmol) in dry CH_3CN (50 mL) was added $BF_3{\cdot}OEt_2$ (650 μL, 5.15 mmol) at 0°C and the reaction mixture was stirred at the same temperature for 1 h. The reaction was quenched with saturated aqueous sodium hydrogen carbonate and the mixture was extracted with EtOAc. The organic layer was washed with water and brine, dried over anhydrous $MgSO_4$, and concentrated in vacuo. The crude product was purified by column chromatography on silica gel (*n*-hexane/EtOAc, 1:1) to give compound **10** as a white solid (1.28 g, 94%). ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 1.24$ (dd, $J = 6.3$ and 12.0 Hz, 1H), 1.97–2.04 (br s, 1H), 2.19 (dd, $J = 12.0$ and 12.0 Hz, 1H), 2.76 (ddddd, $J = 4.8$, 6.0, 6.3, 10.0 and 12.0 Hz, 1H), 3.23 (d, $J = 9.5$ Hz, 1H), 3.43 (d, $J = 9.5$ Hz, 1H), 3.78 (br d, $J = 12.2$ Hz, 1H), 3.86 (br d, $J = 12.2$ Hz, 1H), 4.19 (dd, $J = 10.0$ and 11.4 Hz, 1H), 4.50 (dd, $J = 6.0$ and 11.4 Hz, 1H), 4.86 (d, $J = 12.0$ Hz, 1H), 4.95 (d, $J = 12.3$ Hz, 1H), 5.00 (d, $J = 12.0$ Hz, 1H), 5.07 (d, $J = 12.3$ Hz, 1H), 5.17 (d, $J = 4.8$ Hz, 1H), 7.22–7.38 (m, 10H), 7.43 (dd, $J = 7.2$ and 7.2 Hz, 2H), 7.57 (dt, $J = 0.70$ and 7.2 Hz, 1H), 7.98 ppm (dd, $J = 0.70$ and 7.2 Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 35.2$, 41.0, 46.5, 53.9, 62.2, 64.3, 66.9, 67.0, 89.4, 128.20 (2C), 128.37 (2C), 128.44 (2C), 128.46 (2C), 128.54 (2C), 128.64 (2C), 129.51, 129.56 (2C), 133.22, 135.2, 135.4, 166.2, 169.9, 170.5 ppm; MS (FAB, positive-ion mode, *m*-nitrobenzyl alcohol): calcd for $C_{30}H_{31}O_8$: 531 $[M+H]^+$; found: 531.

*Separation of Enantiomers (−)-(1S,5R)-**10** and (+)-(1R,5S)-**10***

Racemate **10**: (360 mg, 0.678 mmol) was separated by HPLC (CHIRALPAK IA, 250 mm × 20 mm, 5 μm); flow rate: 6.0 mL min^{-1}, mobile phase, *n*-hexane/$CHCl_3$/2-propanol, 65:32:3) to give isomers (1*R*,5*S*)-**10** (140 mg, 0.320 mmol) and (1*S*,5*R*)-**10** (140 mg, 0.320 mmol).

(1*S*,5*R*)-**10**: Colorless oil; $[\alpha]_D^{27} = -21.3$ ($c = 0.72$, $CHCl_3$), $t_r = 37$ min (CHIRALPAK IA, 250 mm × 4.6 mm, 5 μm; flow rate: 1.0 mL min^{-1}, *n*-hexane/$CHCl_3$, 2:1).

(1*R*,5*S*)-**10**: Colorless oil; $[\alpha]_D^{27} = +19.8$ ($c = 1.36$, $CHCl_3$), $t_r = 32$ min (CHIRALPAK IA, 250 mm × 4.6 mm, 5 μm; flow rate: 1.0 mL min^{-1}, *n*-hexane/$CHCl_3$, 2:1).

*Typical Procedure for the Synthesis of Compounds **19a–19j***

To a solution of (1*S*,5*R*)-**10** or (1*R*,5*S*)-**10**, *i*Pr_2NEt (2.0 equiv), and *N*-methylimidazole (3.0 equiv) in CH_2Cl_2 (0.1 M), was added an acid chloride RCOCl (1.5 equiv) at 0°C, and the mixture was stirred for 4 h at RT. The reaction was quenched with saturated sodium hydrogen carbonate and the mixture was extracted with EtOAc. The organic layer was washed with water and brine, dried over anhydrous $MgSO_4$, and concentrated in vacuo. The crude product was purified by column chromatography on silica gel to give (1*S*,5*R*)- or (1*R*,5*S*)-**19a–19j**.

*(1S,2R,3S,4R,5R)-1,5-Bisbenzoyloxymethyl-7-oxa-bicyclo[2.2.1]heptane-2,3-dicarboxylic acid dibenzyl ester ((1S,5R)-**19a**)*

Yield: 42%; $[\alpha]_D^{28} = -47.1$ ($c = 1.6$, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 1.37$ (dd, $J = 6.0$ and 12.5 Hz, 1H), 2.12 (t, $J = 12.1$ and 12.5 Hz, 1H), 2.81 (ddddd, $J = 4.8$, 6.0, 6.5, 9.9, and 12.1 Hz, 1H), 3.34 (d, $J = 9.4$ Hz, 1H), 3.47 (d, $J = 9.4$ Hz, 1H), 4.14 (dd, $J = 9.9$ and 11.5 Hz, 1H), 4.46 (dd, $J = 6.5$ and 11.5 Hz, 1H), 4.56 (d, $J = 11.5$ Hz, 1H), 4.61 (d, $J = 11.5$ Hz, 1H), 4.72 (d, $J = 12.0$ Hz, 1H), 4.84 (d, $J = 12.0$ Hz, 1H), 4.92 (d, $J = 12.1$ Hz, 1H), 5.05 (d, $J = 12.1$ Hz, 1H), 5.22 (d, $J = 4.8$ Hz, 1H), 7.03–7.22 (m, 10H), 7.34 (dd, $J = 7.2$ and 7.2 Hz, 1H), 7.39 (dd, $J = 7.2$ and 7.2 Hz, 2H), 7.51 (t, $J = 7.2$ Hz, 2H), 7.53 (t, $J = 7.2$ Hz, 1H), 7.92 (d, $J = 7.2$ Hz, 2H), 7.94 ppm (d, $J = 7.2$ Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 36.98$, 40.65, 46.75, 54.50, 63.89, 64.28, 67.00 (2C), 79.48, 87.04, 128.27 (2C), 128.33 (2C), 128.39 (2C), 128.49 (2C), 128.41 (4C), 128.49 (2C), 128.55 (4C), 129.47, 129.58, 129.70, 129.74, 133.14, 133.27, 165.63, 166.19, 169.65, 170.11 ppm.

*(1R,2S,3R,4S,5S)-1,5-Bisbenzoyloxymethyl-7-oxa-bicyclo[2.2.1]heptane-2,3-dicarboxylic acid dibenzyl ester ((1R,5S)-**19a**)*

Yield: 72%; $[\alpha]_D^{28} = +49.6$ ($c = 1.6$, $CHCl_3$).

*Typical Procedure for the Synthesis of Compounds **1a–1j***

To a solution of (1*S*,5*R*)- or (1*R*,5*S*)-**19a–19j** in EtOAc (0.1 M) was added 10 wt.% Pd/C (Degussa type, about 10 mg) and the mixture was stirred for 1 h at RT under a hydrogen atmosphere. The mixture was filtered through a membrane filter and the filtrate was concentrated in vacuo. The crude product was dissolved in acetic anhydride (0.1 M) and the reaction mixture was stirred for 4 h at 50°C, concentrated in vacuo, and the residue was recrystallized from Et_2O, CH_2Cl_2, and *n*-hexane to give (1*S*,5*R*)- or (1*R*,5*S*)-**1a–1j**.

*(1S,2R,3S,4R,5R)-1,5-Dibenzoyloxymethyl-7-oxabicyclo[2.2.1]heptane-2,3-dicarboxylic anhydride ((1S,5R)-**1a**)*

Yield: 70%; $[\alpha]_D^{28} = -29.3$ ($c = 2.6$, CH_3CN); ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 1.45$ (dd, $J = 5.9$ and 12.3 Hz, 1H), 2.26 (dd, $J = 12.3$ and 12.3 Hz, 1H), 2.98 (ddddd, $J = 4.9$, 5.9, 6.0, 9.3, and 12.3 Hz, 1H), 3.33 (d, $J = 7.5$ Hz, 1H), 3.78 (d, $J = 7.5$ Hz, 1H), 4.26 (dd, $J = 9.3$ and 12.0 Hz, 1H), 4.54 (dd, $J = 6.0$ and 12.0 Hz, 1H), 4.77 (d, $J = 12.4$ Hz, 1H), 4.89 (d, $J = 12.4$ Hz, 1H), 5.10 (d, $J = 4.9$ Hz, 1H), 7.46 (dd, $J = 7.5$ and 7.5 Hz, 2H), 7.48 (dd, $J = 7.5$ and 7.5 Hz, 2H), 7.59 (t, $J = 7.5$ Hz, 1H), 7.62 (t, $J = 7.5$ Hz, 2H), 8.01 (d, $J = 7.5$ Hz, 1H), 8.04 ppm (d, $J = 7.5$ Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 34.69$, 40.75, 47.63, 51.67, 62.19, 63.54, 81.80, 88.18, 128.41 (2C), 128.55 (2C), 129.05, 129.11, 129.48 (2C), 129.66 (2C), 133.31, 133.48, 165.50, 165.96, 168.49, 170.86 ppm; MS (FAB, positive-ion mode, *m*-nitrobenzyl alcohol): calcd for $C_{24}H_{21}O_8$: 437 $[M+H]^+$; found: 437; elemental analysis calcd (%) for $C_{24}H_{20}O_8$: C 66.05, H 4.62; found: C 65.68, H 4.64.

*(1R,2S,3R,4S,5S)-1,5-Dibenzoyloxymethyl-7-oxabicyclo[2.2.1]heptane-2,3-dicarboxylic anhydride ((1R,5S)-**1a**)*

Yield: 17%; $[\alpha]_D^{28} = +33.5$ ($c = 0.54$, CH_3CN); elemental analysis calcd (%) for $C_{24}H_{20}O_8$: C 66.05, H 4.62; found: C 65.84, H 4.66.

*(1S,2R,3S,4R,5R)-5-Benzoyloxymethyl-1-(2-toluoyloxymethyl)-7-oxa-bicyclo[2.2.1]heptane-2,3-dicarboxylic anhydride ((1S,5R)-**1b**)*

Yield: 73%; $[\alpha]_D^{28} = -29.0$ ($c = 2.6$, CH_3CN); ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 1.45$ (dd, $J = 6.0$ and 12.4 Hz, 1H), 2.28 (dd, $J = 12.4$ and 12.4 Hz, 1H), 2.61 (s, 3H), 2.93 (ddddd, $J = 4.8$, 6.0, 6.4, 9.7, and 12.4 Hz, 1H), 3.34 (d, $J = 7.5$ Hz, 1H), 3.77 (d, $J = 7.5$ Hz, 1H), 4.26 (dd, $J = 9.7$ and 12.0 Hz, 1H), 4.53 (dd, $J = 6.4$ and 12.0, Hz, 1H), 4.72 (d, $J = 12.4$ Hz, 1H), 4.82 (d, $J = 12.4$ Hz, 1H), 5.09 (d, $J = 4.8$ Hz, 1H), 7.20–7.35 (m, 2H), 7.43 (dd, $J = 7.6$ and 7.6 Hz, 1H), 7.47 (dd, $J = 6.8$ and 7.5 Hz, 2H), 7.61 (dd, $J = 7.6$ and 7.6 Hz, 1H), 7.92 (d, $J = 7.6$ Hz, 1H), 8.02 ppm (d, $J = 6.8$ Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 21.89$, 34.81, 40.80, 47.50, 51.78, 62.13, 63.53, 81.88, 88.10, 125.75, 128.46, 128.56 (2C), 129.07, 129.49 (2C), 130.58, 131.71, 132.35, 133.48, 140.49, 165.96, 166.24, 168.40, 170.85 ppm; MS (FAB, positive-ion mode, *m*-nitrobenzyl alcohol): calcd

for $C_{25}H_{23}O_8$: 451 $[M+H]^+$; found: 451; elemental analysis calcd (%) for $C_{25}H_{22}O_8$: C 66.66, H 4.92; found: C 66.49, H 4.94.

*(1R,2S,3R,4S,5S)-5-Benzoyloxymethyl-1-(2-toluoyloxymethyl)-7-oxa-bicyclo[2.2.1]heptane-2,3-dicarboxylic anhydride ((1R,5S)-**1b**)*

Yield: 67%; $[\alpha]_D^{28}=+30.4$ ($c=2.8$, CH_3CN); elemental analysis calcd (%) for $C_{25}H_{22}O_8$: C 66.66, H 4.92; found: C 66.71, H 4.96.

*(1S,2R,3S,4R,5R)-5-Benzoyloxymethyl-1-(3-toluoyloxymethyl)-7-oxa-bicyclo[2.2.1]heptane-2,3-dicarboxylic anhydride ((1S,5R)-**1c**)*

Yield: 26%; $[\alpha]_D^{28}=-29.1$ ($c=0.9$, CH_3CN); ^{1}H NMR (400 MHz, $CDCl_3$): $\delta=1.45$ (dd, $J=6.0$ and 12.4 Hz, 1H), 2.28 (dd, $J=12.4$ and 12.4 Hz, 1H), 2.41 (s, 3H), 2.93 (ddddd, $J=4.8$, 6.0, 6.0, 9.7, and 12.4 Hz, 1H), 3.34 (d, $J=7.5$ Hz, 1H), 3.78 (d, $J=7.5$ Hz, 1H), 4.26 (dd, $J=9.7$ and 12.0 Hz, 1H), 4.53 (dd, $J=6.0$ and 12.0, Hz, 1H), 4.73 (d, $J=12.4$ Hz, 1H), 4.88 (d, $J=12.4$ Hz, 1H), 5.10 (d, $J=4.8$ Hz, 1H), 7.20–7.40 (m, 1H), 7.43 (dd, $J=7.6$ and 7.6 Hz, 1H), 7.47 (dd, $J=6.8$ and 7.5 Hz, 1H), 7.61 (dd, $J=7.6$ and 7.6 Hz, 1H), 7.92 (d, $J=7.6$ Hz, 1H), 7.83 (d, $J=7.9$ Hz, 1H), 7.85 (s, 1H), 8.01 ppm (d, $J=7.9$ Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta=21.37$, 34.65, 40.74, 47.60, 51.69, 62.12, 63.55, 81.81, 88.22, 126.81, 128.30, 128.56 (2C), 129.03, 129.06, 129.49 (2C), 130.18, 133.49, 134.10, 138.23, 165.71, 165.96, 168.45, 170.86 ppm; MS (FAB, positive-ion mode, *m*-nitro benzyl alcohol) calcd for $C_{25}H_{23}O_8$: 451 $[M+H]^+$; found: 451; elemental analysis calcd (%) for $C_{25}H_{22}O_8$: C 66.66, H 4.92; found: C 66.42, H 4.94.

*(1R,2S,3R,4S,5S)-5-Benzoyloxymethyl-1-(3-toluoyloxymethyl)-7-oxa-bicyclo[2.2.1]heptane-2,3-dicarboxylic anhydride ((1R,5S)-**1c**)*

Yield: 64%; $[\alpha]_D^{28}=+28.4$ ($c=2.5$, CH_3CN); elemental analysis calcd (%) for $C_{25}H_{22}O_8{\cdot}0.2H_2O$: C 66.13, H 4.97; found: C 66.05, H 4.89.

*(1S,2R,3S,4R,5R)-5-Benzoyloxymethyl-1-(4-toluoyloxymethyl)-7-oxa-bicyclo[2.2.1]heptane-2,3-dicarboxylic anhydride ((1S,5R)-**1d**)*

Yield: 50%; $[\alpha]_D^{28}=-28.3$ ($c=1.6$, CH_3CN); ^{1}H NMR (400 MHz, $CDCl_3$): $\delta=1.44$ (dd, $J=6.0$ and 12.0 Hz, 1H), 2.26 (dd, $J=12.0$ and 12.0 Hz, 1H), 2.42 (s, 3H), 2.92 (ddddd, $J=4.8$, 6.0, 6.0, 9.1, and 12.0 Hz, 1H), 3.32 (d, $J=7.6$ Hz, 1H), 3.77 (d, $J=7.6$ Hz, 1H), 4.25 (dd, $J=9.1$ and 12.0 Hz, 1H), 4.53 (dd, $J=6.0$ and 12.0 Hz, 1H), 4.74 (d, $J=12.4$ Hz, 1H), 4.87 (d, $J=12.4$ Hz, 1H), 5.09 (d, $J=4.8$ Hz, 1H), 7.25 (d, $J=8.0$ Hz,, 2H), 7.48 (dd, $J=7.6$ and 7.6 Hz, 2H), 7.61 (t, $J=7.6$ Hz, 1H), 7.93 (d, $J=8.0$ Hz, 2H), 8.02 ppm (d, $J=7.6$ Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta=21.80$, 34.72, 40.75, 47.61, 51.68, 62.00, 63.55, 81.81, 88.28, 126.37, 128.57 (2C), 129.07, 129.13 (2C), 129.50 (2C), 129.72 (2C), 133.49, 144.12, 165.58, 165.96, 168.41, 170.84 ppm; MS (FAB, positive-ion mode, *m*-nitrobenzyl alcohol) calcd for $C_{25}H_{23}O_8$: 451 $[M+H]^+$; found: 451; elemental analysis calcd (%) for $C_{25}H_{22}O_8{\cdot}0.2H_2O$: C 66.13, H 4.97; found: C 66.15, H 5.04.

*(1R,2S,3R,4S,5S)-5-Benzoyloxymethyl-1-(4-toluoyloxymethyl)-7-oxa-bicyclo[2.2.1]heptane-2,3-dicarboxylic anhydride ((1R,5S)-**1d**)*

Yield: 41%; $[\alpha]_D^{28}=+27.8$ ($c=1.2$, CH_3CN); elemental analysis calcd (%) for $C_{25}H_{22}O_8$: C 66.66, H 4.92; found: C 66.62, H 4.96.

*(1S,2R,3S,4R,5R)-5-Benzoyloxymethyl-1-cyclohexanecarbonyloxymethyl-7-oxa-bicyclo[2.2.1]heptane-2,3-dicarboxylic anhydride ((1S,5R)-**1e**)*

Yield: 67%; $[\alpha]_D^{28}=-27.1$ ($c=1.5$, CH_3CN); ^{1}H NMR (400 MHz, $CDCl_3$): $\delta=1.21$–1.50 (m, 6H), 1.51–1.80 (m, 3H), 1.83–1.98 (m, 2H), 2.15 (dd, $J=12.2$ and 12.2 Hz, 1H), 2.37 (tt, $J=3.6$ and 11.1 Hz, 1H), 2.89 (ddddd, $J=4.8$, 6.0, 6.3, 9.5, and 12.2 Hz, 1H), 3.25 (d, $J=7.6$ Hz, 1H), 3.74 (d, $J=7.6$ Hz, 1H), 4.23 (dd, $J=9.5$ and 11.9 Hz, 1H), 4.48 (d, $J=12.7$ Hz, 1H), 4.54 (dd, $J=6.0$ and 11.9 Hz, 1H), 4.58 (d, $J=12.7$ Hz, 1H), 5.06 (d, $J=4.8$ Hz, 1H), 7.48 (dd, $J=7.6$ and 8.4 Hz,, 2H), 7.61 (t, $J=7.6$ Hz, 1H), 8.00 ppm (d, $J=8.4$ Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta=$ 25.41, 25.44, 25.74, 28.93, 29.05, 34.69, 40.71, 42.95, 47.54, 51.58, 61.60, 63.53, 81.77, 88.08, 128.57 (2C), 129.07, 129.49 (2C), 133.49, 165.96, 168.35, 170.86, 174.86 ppm; MS (FAB, positive-ion mode, *m*-nitrobenzyl alcohol) calcd for $C_{24}H_{27}O_8$: 443 $[M+H]^+$; found: 443; elemental analysis calcd (%) for $C_{24}H_{26}O_8$: C 65.15, H 5.92; found: C 65.22, H 5.93.

*(1R,2S,3R,4S,5S)-5-Benzoyloxymethyl-1-cyclohexanecarbonyloxymethyl-7-oxa-bicyclo[2.2.1]heptane-2,3-dicarboxylic anhydride ((1R,5S)-**1e**)*

Yield: 80%; $[\alpha]_D^{28}=+26.9$ ($c=2.0$, CH_3CN); elemental analysis calcd (%) for $C_{24}H_{26}O_8$: C 65.15, H 5.92; found: C 64.93, H 5.93.

*(1S,2R,3S,4R,5R)-5-Benzoyloxymethyl-1-cyclopentanecarbonyloxymethyl-7-oxa-bicyclo[2.2.1]heptane-2,3-dicarboxylic anhydride ((1S,5R)-**1f**)*

Yield: 76%; $[\alpha]_D^{28}=-29.4$ ($c=2.2$, CH_3CN); ^{1}H NMR (400 MHz, $CDCl_3$): $\delta=1.38$ (dd, $J=6.0$ and 12.4 Hz, 1H), 1.57–1.90 (m, 8H), 2.15 (dd, $J=12.4$ and 12.4 Hz, 1H), 2.89 (tt, $J=8.0$ and 8.0 Hz, 1H), 2.90 (ddddd, $J=4.8$, 6.0, 6.0, 9.5, and 12.4 Hz, 1H), 3.25 (d, $J=7.6$ Hz, 1H), 3.74 (d, $J=7.6$ Hz, 1H), 4.24 (dd, $J=9.5$ and 11.5 Hz, 1H), 4.48 (d, $J=12.7$ Hz, 1H), 4.55 (dd, $J=6.0$ and 11.5 Hz, 1H), 4.58 (d, $J=12.7$ Hz, 1H), 5.06 (d, $J=4.8$ Hz, 1H), 7.48 (dd, $J=7.6$ and 8.4 Hz,, 2H), 7.61 (t, $J=7.6$ Hz, 1H), 8.01 ppm (d, $J=8.4$ Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta=25.83$ (2C), 29.98, 30.08, 39.88, 40.29, 43.98, 48.30, 51.98, 61.79, 63.67, 80.89, 88.04, 128.38 (2C), 129.04, 129.52 (2C), 133.65, 165.95, 168.39, 170.91, 175.61 ppm; MS (FAB, positive-ion mode, *m*-nitrobenzyl alcohol) calcd for $C_{23}H_{25}O_8$: 429 $[M+H]^+$; found: 429; elemental analysis calcd (%) for $C_{23}H_{24}O_8$: C 64.48, H 5.62; found: C 64.54, H 5.68.

*(1R,2S,3R,4S,5S)-5-Benzoyloxymethyl-1-cyclopentanecarbonyloxymethyl-7-oxa-bicyclo[2.2.1]heptane-2,3-dicarboxylic anhydride ((1R,5S)-**1f**)*

Yield: 64%; $[\alpha]_D^{28}=+27.1$ ($c=2.1$, CH_3CN); elemental analysis calcd (%) for $C_{23}H_{24}O_8$: C 64.48, H 5.62; found: C 64.41, H 5.66.

*(1S,2R,3S,4R,5R)-5-Benzoyloxymethyl-1-cyclopropanecarbonyloxymethyl-7-oxa-bicyclo[2.2.1]heptane-2,3-dicarboxylic anhydride ((1S,5R)-**1g**)*

Yield: 95%; $[\alpha]_D^{28}=-30.9$ ($c=2.3$, CH_3CN); ^{1}H NMR (400 MHz, $CDCl_3$): $\delta=0.89$–0.94 (m, 2H), 1.03–1.06 (m, 2H), 1.38 (dd, $J=6.0$ and 12.2 Hz, 1H), 1.66 (dddd, $J=4.3$, 4.3, 4.3, and 4.3 Hz, 1H), 2.19 (dd, $J=12.2$ and 12.2 Hz, 1H), 2.90 (ddddd, $J=4.8$, 6.0, 6.0, 9.2, and 12.2 Hz, 1H), 3.25 (d, $J=7.6$ Hz, 1H), 3.74 (d, $J=7.6$ Hz, 1H), 4.24 (dd, $J=9.2$ and 11.5 Hz, 1H), 4.49 (d, $J=12.3$ Hz, 1H), 4.52 (dd, $J=6.0$ and 11.5 Hz, 1H), 4.58 (d, $J=12.3$ Hz, 1H), 5.06 (d, $J=4.8$ Hz, 1H), 7.48 (dd, $J=7.6$ and 7.8 Hz,, 2H), 7.61 (t, $J=7.6$ Hz, 1H), 8.01 ppm (d, $J=7.8$ Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta=9.01$ (2C), 12.73, 34.44, 40.68, 47.57, 51.58, 61.80, 63.53, 81.76, 88.04, 128.55 (2C), 129.06, 129.47 (2C), 133.48, 165.95, 168.41, 170.88, 173.86 ppm; MS (FAB, positive-ion mode, *m*-nitrobenzyl alcohol): calcd for $C_{21}H_{21}O_8$: 401 $[M+H]^+$; found: 401; elemental analysis calcd (%) for $C_{21}H_{20}O_8$: C 63.00, H 5.03; found: C 63.03, H 5.08.

*(1R,2S,3R,4S,5S)-5-Benzoyloxymethyl-1-cyclopropanecarbonyloxymethyl-7-oxa-bicyclo[2.2.1]heptane-2,3-dicarboxylic anhydride ((1R,5S)-**1g**)*

Yield: 94%; $[\alpha]_D^{28}=+31.5$ ($c=2.6$, CH_3CN); elemental analysis calcd (%) for $C_{21}H_{20}O_8$: C 63.00, H 5.03; found: C 62.72, H 5.04.

*(1S,2R,3S,4R,5R)-5-Benzoyloxymethyl-1-(2,2-dimethyl-propionyloxymethyl)-7-oxa-bicyclo[2.2.1]heptane-2,3-dicarboxylic anhydride ((1S,5R)-**1h**)*

Yield: 32%; $[\alpha]_D^{28}=-28.0$ ($c=0.7$, CH_3CN); ^{1}H NMR (400 MHz, $CDCl_3$): $\delta=1.24$ (s, 9H), 1.38 (dd, $J=5.5$ and 12.2 Hz, 1H), 2.15 (dd, $J=12.2$ and 12.2 Hz, 1H), 2.90 (ddddd, $J=4.8$, 5.5, 5.5, 9.5, and 12.2 Hz, 1H), 3.23 (d, $J=7.6$ Hz, 1H), 3.74 (d, $J=7.6$ Hz, 1H), 4.23 (dd, $J=9.5$ and 11.9 Hz, 1H), 4.49 (d, $J=12.8$ Hz, 1H), 4.54 (dd, $J=5.5$ and 11.9 Hz, 1H), 4.58 (d, $J=12.0$ Hz, 1H), 5.07 (d, $J=4.8$ Hz, 1H), 7.49 (dd, $J=7.2$ and 7.6 Hz, 2H), 7.62 (t, $J=7.6$ Hz, 1H), 8.02 ppm (d, $J=7.2$ Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta=27.18$ (3C), 34.93, 38.97, 40.73, 47.55, 51.57, 61.91, 63.53, 81.74, 88.16, 128.57 (2C), 129.06, 129.50 (2C), 133.52, 165.96 168.25, 170.81, 177.30 ppm; MS (FAB, positive-ion mode, *m*-nitrobenzyl alcohol): calcd for $C_{22}H_{25}O_8$: 417 $[M+H]^+$; found: 417; elemental analysis calcd (%) for $C_{22}H_{24}O_8{\cdot}0.2H_2O$: C 62.91, H 5.86; found: C 62.79, H 5.96.

*(1R,2S,3R,4S,5S)-5-Benzoyloxymethyl-1-(2,2-dimethyl-propionyloxymethyl)-7-oxa-bicyclo[2.2.1]heptane-2,3-dicarboxylic anhydride ((1R,5S)-**1h**)*

Yield: 28%; $[\alpha]_D^{28} = +27.4$ ($c = 0.7$, CH_3CN); elemental analysis calcd (%) for $C_{22}H_{24}O_8$: C 63.45, H 5.81; found: C 63.29, H 5.85.

*(1S,2R,3S,4R,5R)-5-Benzoyloxymethyl-1-isobutyryloxymethyl-7-oxa-bicyclo[2.2.1]heptane-2,3-dicarboxylic anhydride ((1S,5R)-**1i**)*

Yield: 64%; $[\alpha]_D^{28} = -27.8$ ($c = 2.2$, CH_3CN); ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 1.19$ (d, $J = 7.0$ Hz, 3H), 1.20 (d, $J = 7.0$ Hz, 3H), 1.39 (dd, $J = 6.0$ and 13.1 Hz, 1H), 2.15 (dd, $J = 13.1$ and 13.1 Hz, 1H), 2.62 (qq, $J = 7.0$ and 7.0 Hz, 1H), 2.87–2.93 (m, 1H), 3.25 (d, $J = 7.6$ Hz, 1H), 3.75 (d, $J = 7.6$ Hz, 1H), 4.24 (dd, $J = 9.1$ and 11.4 Hz, 1H), 4.47 (d, $J = 12.3$ Hz, 1H), 4.52 (dd, $J = 6.0$ and 11.4 Hz, 1H), 4.58 (d, $J = 12.3$ Hz, 1H), 5.06 (d, $J = 5.2$ Hz, 1H), 7.48 (dd, $J = 7.6$ and 8.4 Hz,, 2H), 7.61 (t, $J = 7.6$ Hz, 1H), 8.02 ppm (d, $J = 8.4$ Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 18.91$, 19.02, 33.88, 34.72, 40.71, 47.60, 51.55, 61.75, 63.53, 81.73, 88.07, 128.56 (2C), 129.06, 129.47 (2C), 133.49, 165.95, 168.38, 170.87, 175.92 ppm; MS (FAB, positive-ion mode, *m*-nitrobenzyl alcohol): calcd for $C_{21}H_{23}O_8$ 403: $[M+H]^+$; found: 403; elemental analysis calcd (%) for $C_{21}H_{22}O_8$: C 62.68, H 5.51; found: C 62.73, H 5.53.

*(1R,2S,3R,4S,5S)-5-Benzoyloxymethyl-1-isobutyryloxymethyl-7-oxa-bicyclo[2.2.1]heptane-2,3-dicarboxylic anhydride ((1R,5S)-**1i**)*

Yield: 79%; $[\alpha]_D^{28} = +28.9$ ($c = 1.7$, CH_3CN); elemental analysis calcd (%) for $C_{21}H_{22}O_8$: C 62.68, H 5.51; found: C 62.59, H 5.52.

*(1S,2R,3S,4R,5R)-1-Acetoxymethyl-5-benzoyloxymethyl-7-oxa-bicyclo[2.2.1]heptane-2,3-dicarboxylic anhydride ((1S,5R)-**1j**)*

Yield: 65%; $[\alpha]_D^{28} = -13.1$ ($c = 0.82$, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 1.38$ (dd, $J = 5.8$ and 12.8 Hz, 1H), 2.13 (s, 3H), 2.17 (dd, $J = 12.5$ and 12.5 Hz, 1H), 2.81 (ddddd, $J = 4.9$, 6.0, 6.3, 10.8, and 12.6 Hz, 1H), 3.24 (d, $J = 7.5$ Hz, 1H), 3.76 (d, $J = 7.5$ Hz, 1H), 4.24 (dd, $J = 9.4$ and 11.8 Hz, 1H), 4.51 (d, $J = 12.3$ Hz, 1H), 4.52 (dd, $J = 6.0$ and 11.8 Hz, 1H), 4.58 (d, $J = 12.3$ Hz, 1H), 5.07 (d, $J = 4.9$ Hz, 1H), 7.49 (dd, $J = 7.5$ and 7.5 Hz, 2H), 7.62 (t, $J = 7.5$ Hz, 1H), 8.01 ppm (d, $J = 7.5$ Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 20.70$, 34.64, 40.71, 47.60, 51.53, 61.92, 63.48, 81.80, 87.95, 128.59 (2C), 129.03, 129.50 (2C), 133.54, 165.96, 168.33, 169.91, 170.72 ppm; elemental analysis calcd (%) for $C_{19}H_{18}O_8 \cdot 0.2\,H_2O$: C 60.38, H 4.91; found: C 60.42, H 4.85.

*(1R,2S,3R,4S,5S)-1-Acetoxymethyl-5-benzoyloxymethyl-7-oxa-bicyclo[2.2.1]heptane-2,3-dicarboxylic anhydride ((1R,5S)-**1j**)*

Yield: 60%; $[\alpha]_D^{27} = +14.7$ ($c = 0.38$, $CHCl_3$); purity > 97% (HPLC).

*(1S,2R,3S,4R,5R)-1,5-Dibenzoyloxymethyl-7-oxabicyclo[2.2.1]heptane-2,3-N-4-bromophenyldicarboximide ((1S,5R)-**20**)*

To a solution of compound (1*S*,5*R*)-**1a** (48 mg, 0.11 mmol), Et_3N (31 μL, 0.22 mmol), and DMAP (1.2 mg, 0.01 mmol) in toluene (1 mL) was added 4-bromoaniline (29 mg, 0.17 mmol) and the mixture was stirred for 24 h at 130 °C. The mixture was concentrated in vacuo. The crude product was purified by column chromatography on silica gel (*n*-hexane/EtOAc, 2:1) to give the title compound as a white solid (48 mg, 66%). $[\alpha]_D^{28} = +33.5$ ($c = 0.54$, CH_3CN); ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 1.49$ (dd, $J = 5.6$ and 12.3 Hz, 1H), 2.33 (dd, $J = 12.3$ and 12.3 Hz, 1H), 2.96 (ddddd, $J = 5.1$, 5.4, 5.6, 9.0, and 12.3 Hz, 1H), 3.21 (d, $J = 7.3$ Hz, 1H), 3.58 (d, $J = 7.3$ Hz, 1H), 4.31 (dd, $J = 9.0$ and 11.7 Hz, 1H), 4.58 (dd, $J = 5.4$ and 11.7 Hz, 1H), 4.76 (d, $J = 12.7$ Hz, 1H), 4.89 (d, $J = 12.7$ Hz, 1H), 5.07 (d, $J = 5.1$ Hz, 1H), 7.15–7.17 (m, 2H), 7.36–7.62 (m, 8H), 8.00–8.05 ppm (m, 4H); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 35.11$, 40.88, 46.45, 51.19, 62.50, 63.87, 81.23, 87.54, 122.53, 127.74 (2C), 128.30 (2C), 128.50 (2C), 129.23, 129.32, 129.50 (2C), 129.63 (2C), 130.43, 132.16 (2C), 133.16, 133.36, 165.63, 166.01, 173.19, 175.41 ppm; MS (MALDI-TOF, positive ion, α-cyano-4-hydroxycinnamic acid): calcd for $C_{30}H_{25}BrNO_7$: 590.08 $[M+H]^+$; found: 590.24.

*(1S,2R,3S,4R,5R)-1,5-Bisbenzoyloxymethyl-7-oxa-bicyclo[2.2.1]heptane-2,3-dicarboxylic acid dimethyl ester ((1S,5R)-**21a**)*

To a solution of compound (1*S*,5*R*)-**1a** (13.9 mg, 0.032 mmol) in THF (0.5 mL) and MeOH (0.5 mL) was added a solution of trimethylsilyldiazomethane (48 μL, 0.096 mmol, 2.0 M in Et_2O) at RT. The mixture was stirred for 3 h and then concentrated in vacuo. The crude product was purified by preparative thin layer chromatography (*n*-hexane/EtOAc, 4:1) to give the title compound as an amorphous white solid (10.9 mg, 71%). $[\alpha]_D^{28} = -34.4$ ($c = 1.1$, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 1.42$ (dd, $J = 6.0$ and 12.4 Hz, 1H), 2.20 (t, $J = 12.4$ and 12.4 Hz, 1H), 2.86 (ddddd, $J = 4.8$, 6.0, 6.5 9.9, and 12.4 Hz, 1H), 3.33 (d, $J = 9.6$ Hz, 1H), 3.46 (d, $J = 9.6$ Hz, 1H), 3.64 (s, 3H), 3.67 (s, 3H), 4.23 (dd, $J = 9.9$ and 11.0 Hz, 1H), 4.51 (dd, $J = 6.2$ and 11.0 Hz, 1H), 4.61 (d, $J = 11.5$ Hz, 1H), 4.71 (d, $J = 11.5$ Hz, 1H), 5.20 (d, $J = 4.8$ Hz, 1H), 7.34–7.62 (m, 7H), 8.01 (d, $J = 7.2$ Hz, 1H), 8.04 ppm (d, $J = 7.2$ Hz, 2H); HRMS (FAB, positive-ion mode, *m*-nitrobenzyl alcohol): calcd for $C_{26}H_{26}NaO_9$: 505.1475 $[M+Na]^+$; found: 505.1475; purity > 98% (HPLC).

*(1R,2S,3R,4S,5S)-1,5-Bisbenzoyloxymethyl-7-oxa-bicyclo[2.2.1]heptane-2,3-dicarboxylic acid dimethyl ester ((1R,5S)-**21a**)*

Yield: 93%; $[\alpha]_D^{28} = +30.7$ ($c = 1.3$, $CHCl_3$); HRMS (FAB, positive-ion mode, *m*-nitrobenzyl alcohol): calcd for $C_{26}H_{26}NaO_9$: 505.1475 $[M+Na]^+$; found: 505.1451; purity > 99% (HPLC).

Construction of the Binding Model

To construct the binding model of NCA-01 carboxylate and the catalytic site of PP2B, a computational docking study was performed based on the reported PP2B-FKBP-FK506 complex structure (pdb code, 1TCO). The preliminary binding models were constructed in the Affinity module of DS 1.2 (Accelrys). The structures were then subjected to energy-minimization based on molecular dynamics.

Protein Phosphatase Assay

Phosphatases PP1 (rabbit skeletal muscle) and PP2A (rabbit skeletal muscle, composed of α, β, and catalytic subunits) were purchased from UBI (Upstate Biotechnology Inc.). Calmodulin (human brain) and PP2B (human, recombinant) were purchased from CALBIOCHEM. Protein phosphatase assays were carried out according to the UBI procedure in the presence or absence of an appropriate concentration of compound. For PP1 and PP2A assays, the Ser/Thr Phosphatase Assay Kit 1 (UBI) was used, in which free phosphate ions that were released from the substrate phosphopeptide (KRpTIRR) were quantified by colorimetric analysis (630 nm) by using the Malachite Green Method (enzyme concentrations: 4 units mL^{-1} PP1; 1.48 units mL^{-1} PP2A). For the PP2B inhibition assays, RII phosphopeptide (DLDVPIPGRFDRRVpSVAAE, BIOMOL) was used as the substrate. Briefly, the phosphopeptide (90 μM) was incubated with PP2B (1.6 U μL^{-1}) at 30 °C (in 40 mM Tris-HCl, pH 7.5, 100 mM KCl, 6 mM $MgCl_2$, 0.1 mM $CaCl_2$, 0.05 mM DTT, 0.1 mg mL^{-1} BSA, 70 nM calmodulin) in the absence or presence of inhibitors.

IL-2 Production Assay

Jurkat cells (6×10^6 cells mL^{-1}) were placed in a 96-well microplate (145 μL/well) and incubated for 2 h. Test compound (6 mM in DMSO, 0.75 μL) and PHA (Sigma, 7.5 μg/well) were added to each well. The plate was incubated in a CO_2 incubator for 36 h, then the supernatant was collected by centrifugation (400 *g*, 23 °C for 5 min). The concentration of IL-2 in each supernatant (50 μL) was measured by using the human IL-2 ELISA system (Easy ELISA IL-2 human, GE Healthcare). OD values at 450 nm were measured with a microplate reader (SpectraMax M2, Molecular Devices).

Cell Viability

Jurkat cells were incubated in the presence of a test compound and PHA under the same conditions as that used for the IL-2-production assay, and the cell viability was measured by using AlamarBlue reagent (Biosource International; Ex. 560 nm, Em. 590 nm; SpectraMax M2, Molecular Devices).

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[21] CCDC 656631 ((1*S*,5*R*)-**20**) contains the supplementary crystallographic data for this paper. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif.

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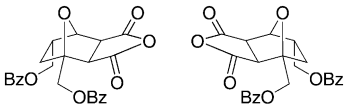
FULL PAPERS

Protein phosphatase 2B inhibitor: Optically pure norcantharidin analogue NCA-01, a highly selective inhibitor of protein phosphatase 2B (PP2B), has been synthesized. The PP2B-inhibitory activity of NCA-01 and its derivatives were independent of the enantiomeric form.



(*1S*, *5R*)-NCA-01 (*1R*, *5S*)-NCA-01

Protein Inhibitors

*Tadashi Shimizu, Masato Iizuka, Hiroko Matsukura, Daisuke Hashizume, Mikiko Sodeoka** ____ ▮▮▮▮–▮▮▮▮

Synthesis of Optically Pure Norcantharidin Analogue NCA-01, a Highly Selective Protein Phosphatase 2B Inhibitor, and its Derivatives

