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Synthesis and HIV-1 integrase inhibitory activity of spiroundecane(ene) derivatives

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Abstract—Fifteen 2,4-dioxaspiro[5.5]undecane ketone and 2,4-dioxa-spiro[5.5]undec-8-ene (spiroundecane(ene)) derivatives were synthesized using the Diels–Alder reaction. Inhibition of human immunodeficiency virus integrase (IN) was examined. Eight spiroundecane(ene) derivatives inhibited both 3′-processing and strand transfer reactions catalyzed by IN. SAR studies showed that the undecane core with at least one furan moiety is preferred for IN inhibition. Moreover, crosslinking experiments showed that spiroundecane derivatives did not affect IN–DNA binding at concentrations that block IN catalytic activity, indicating spiroundecane derivatives inhibit preformed IN–DNA complex. The moderate toxicity of spiroundecane(ene) derivatives encourages the further design of therapeutically relevant analogues based on this novel chemotype of IN inhibitors.

Human immunodeficiency virus (HIV) enzymes are targets for antiretroviral therapy due to their requirement for the HIV life cycle. At the present time, only inhibitors of HIV reverse transcriptase and HIV protease are approved for AIDS therapy.[1] However, the third viral enzyme—integrase (IN) is also a promising target because of its non-homology to mammalian enzymes.[2] In contrast, toxicity of compounds that inhibit HIV reverse transcriptase and protease is believed to be due to their homology to the host cell's enzymes. Promising results of clinical trials for two new IN inhibitors—a derivative from quinolone antibiotics (JTK-303/GS-9137, Gilead Sciences, Inc.) and the compound MK-0518 from 'Merck & Co' were announced recently, providing the proof of concept for IN inhibitors as antiretroviral therapy.[3]

The joining (integration) of the viral cDNA to host cellular DNAs is performed by IN whose catalytic site is characterized by the D,D-35-E motif.[4] The first IN-catalyzed reaction, 3′-processing (3′-P), consists of the cleavage of the viral cDNA immediately 3′ from the conserved CA-sequences at the 3′-ends of the HIV long terminal repeats (LTRs). 3′-P occurs in the cytoplasm after viral reverse transcription. It is still unclear whether 3′-P takes place before or after preintegration complex (PIC) formation and whether PIC formation requires the catalytic activity of IN. As almost all viral cDNA within the PICs consists of 3′-processed ends and viral DNA is not protected from nucleases after isolation of PICs with mutant IN,[5] 3′-P probably precedes and may be required for the formation of PICs. The second IN-catalyzed reaction, strand transfer (ST), consists of joining of viral cDNA to cellular DNA. ST is therefore contingent of the 3′P and migration of the PIC into the nucleus.

Recently the development of HIV integration inhibitors has focused on inhibitors of the ST reaction.[3] However, equal importance of 3′-P for HIV integration as well as its possible involvement in PIC formation make 3′-P a rational approach to inhibit HIV integration. It might also be logical to combine 3′-P inhibitors with the currently developed ST inhibitors.

In our systematic search for novel IN inhibitors, we have identified spirocyclic ketone derivatives (Scheme 1) as compounds that effectively block recombinant HIV IN. Spirocyclic ketones are employed as intermediates in

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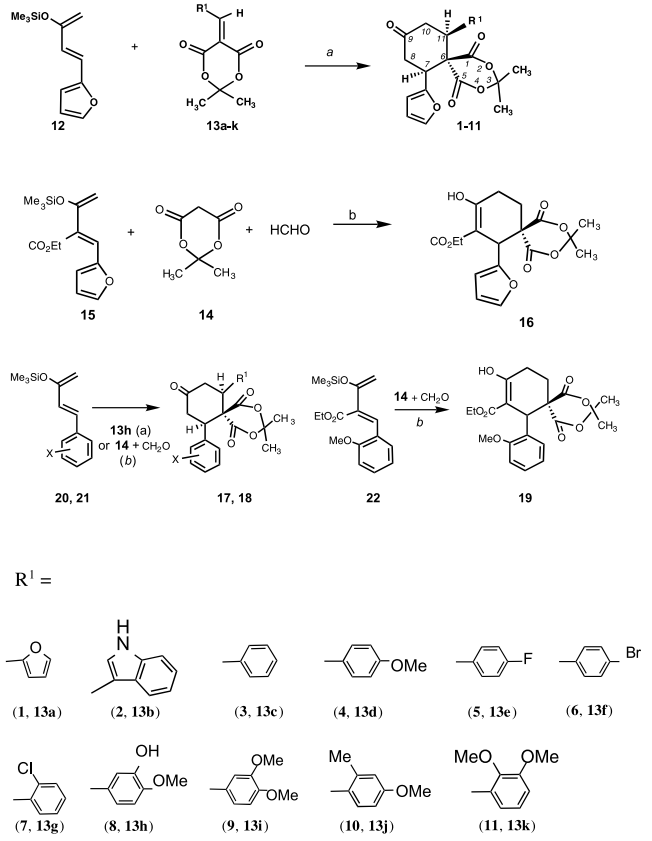

Scheme 1. Reagents and conditions: (a) L-proline (0.2 mmol), MeCN, rt, 13–35 h; (b) L-proline (0.2 mmol), MeCN, rt, 1.5 h.

the total stereoselective synthesis of natural products such as gymnodimine (marine toxin from oysters) and laxaphycins A (cytotoxic compounds from marine cyanobacterium).[6]

The synthesis of the above compounds **1**–**11** is outlined in Scheme 1. Diels–Alder reaction of 1-(2-furyl)-3-trimethylsiloxy-butadiene **12**[7] and 5-aryl(hetaryl)methylene-2,2-dimethyl-1,3-dioxane-4,6-diones (**13a**–**k**) with a catalytic amount of L-proline in acetonitrile at ambient temperature proceeds in the regioselective fashion and furnished the corresponding spirodioxane triones (**1**–**11**) (yield 63–92%).[8] Compound **4** was also obtained by the three-component reaction of diene **12**, 4-methoxy-benzaldehyde, and Meldrum's acid **14** in the presence of L-proline in methanol solution (yield 56%). The reaction of 1-(2-furyl)-2-ethoxycarbonyl-3-trimethylsiloxy-butadiene **15**[9] with methylene Meldrum's acid (**13l** R^1 = H) (obtained in situ from Meldrum's acid **14** and formaldehyde with L-proline in equivalent acetonitrile) gave 7-(furan-2-yl)-9-hydroxy-3,3-dimethyl-1,5-dioxa-spiro[5,5]undec-8-ene-8-carboxylic acid ethyl esters **16**.[10] Compounds **17**–**19**,[10] containing aryl substituents in the C-7 position, were obtained as follows. Cycloaddition reaction of 1-(2-methoxyphenyl)-3-trimethylsiloxy-butadiene **20**[11] with 5-[1-(3-hydroxy-4-methoxy-phenyl)-ethylidene]-2,2-dimethyl-[1,3]dioxane-4,6-dione **13h** leads to compound **17**. By three-component reaction of 1-(4-methoxyphenyl)-2-ethoxycarbonyl-3-trimethylsiloxy-butadiene **21**[9] with Meldrum's acid and formaldehyde compound **18** was obtained. The reaction of 1-(2-methoxyphenyl)-2-ethoxycarbonyl-3-trimethylsiloxy-butadiene **22**[11] with Meldrum's acid and formaldehyde yielded the dioxaspiro-undec-8-ene derivative **19**.

All compounds were formed as single diastereomers. The stereochemistry of products was established by NMR analysis. Relative stereochemistry of cyclohexanone derivatives **1**–**11** and **17** was determined by analysis of the vicinal coupling constants for protons at C-7 and C-11. The syn-arrangement of aryl(hetaryl) and furyl substituents follows from the axial–axial coupling constants between 7-H and 8-H (J = 13.4–14.8 Hz) and 10-H and 11H (J = 13.3–15.0 Hz). The axial–axial coupling constants between 7-H and 8-H were also observed in the case of compound **18**.

The structure of compound **1** was determined by single-crystal X-ray diffraction analysis (Fig. 1[12]). The bond lengths in the molecule are close to the statistical mean values. In the Cambridge structural database (University of Cambridge, UK. Version 5.26) we found only two compounds[6a,13] in which the cyclohexane ring was spiro fused to the 1,3-dioxane ring. The most closest structural analogue was 3,3-dimethyl-7-(4-nitrophenyl)-11-phenyl-2,4-dioxaspiro[5,5]undecane-1,5,9-trione.[6a]

All compounds were tested against recombinant IN using a 21 bp substrate that allows determination of 3′-P, as the release of the terminal dinucleotide, and ST, as the generation of DNA molecules larger than the starting substrate (Fig. 2 and Table 1).[14] Substitutions on the R^1 position of the spiroundecane core (Scheme 1, Table 1) highlight the importance of this position for IN inhibition. The most active spiroundecane ketone derivative (**2**) contains 3-indolyl moiety at R^1, and is approximately twice more active than the symmetric 7,11-bis-furan-2yl substituted compound (**1**). Substitution to the dimethoxy-phenyl moiety (**11**) at R^1 failed to increase potency compared to the symmetrical compound (**1**). Comparison of four compounds with different substitutions on the phenyl ring (**11**, **9**,

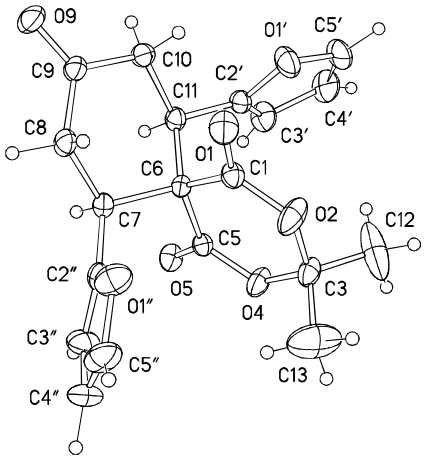

Figure 1. Single-crystal X-ray structure of 3,3-dimethyl-7,11-bis(furan-2-yl)-2,4-dioxa-spiro[5,5]undecane-1,5,9-trione (compound **1**).

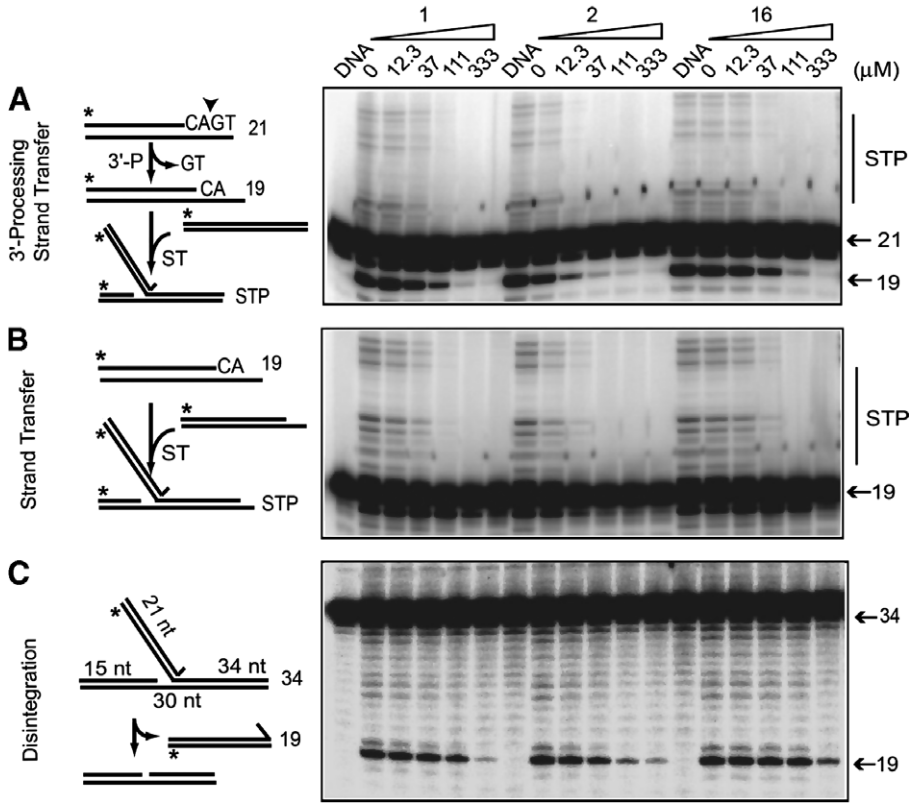

Figure 2. Inhibition of HIV-1 IN 3′-P, ST, and disintegration activities by spiroundecane(ene) derivatives (A) left: schematic representation of the integrase reactions using the 21 bp oligonucleotide duplex that corresponds to the terminal U5 sequence of the HIV-1 LTR. Arrowhead represents the 3′-P site. The initial step involves cleavage of two bases from the 3′-end resulting in a 19 bp product. ST products (STP) result from the covalent joining of the 3′-processed duplex into another identical duplex that serves as the target DNA. Right: PAGE analysis of IN inhibition by investigated derivatives. (B) Left: schematic representation of the ST assay using the preprocessed (19/21) oligonucleotide duplex. Right: PAGE analysis of IN inhibition by the indicated compounds. (C) Left: schematic representation of the disintegration reaction with a Y-oligomer substrate. The disintegration product results from cleavage of the 34-mer oligonucleotide, allowing accumulation of radiolabeled 19-bp oligonucleotide. Right: PAGE analysis of the IN-mediated disintegration reactions in the presence of the indicated compounds. Drug concentrations are shown above each lane. Asterisks represent the 5′-[^{32}P]-label.

Table 1. Inhibition of HIV-1 IN by spiroundecane(ene) derivatives using 21 bp duplex as a substrate

Compound	3-P IC_{50}[a] (μM)	ST IC_{50}[a] (μM)
2	11.1 ± 6.0	9.6 ± 1.6
1	32.9 ± 8.1	17.6 ± 5.9
11	44.8 ± 18.3	44.2 ± 16.8
16	67.8 ± 9.5	43.8 ± 2.8
9	111.3 ± 22.4	91.5 ± 15.7
4	130.5 ± 50.5	36.3 ± 8.5
3	141.1 ± 17.4	131.7 ± 10.9
18	142.3 ± 37.6	97.5 ± 25.4
19	na	281.0 ± 41.3
6	na	na
7	na	na
5	na	na
10	na	na
17	na	na
8	na	na

[a] All data represent mean values and standard deviations for at least three independent experiments (na, not active).

4, **3**) demonstrates the importance of the *ortho*-methoxy substitution in compound **11** for 3′-P inhibition. Additionally, halogen (**5**–**7**) or *para*-hydroxy (**17** and **8**) substitutions to R^1 tend to decrease inhibitory potency with exception for compound **4**.

We tested two spiroundecene derivatives (**16** and **19**) and found that the furan moiety is preferred for IN inhibition compared with methoxyphenyl for the spiroundecene core (compare **16** and **19**). We also found that the spiroundecane core is more potent as a scaffold for IN inhibitors than the spiroundecene core (compare **18** and **19**) (Table 1).

To characterize IN inhibition by spiroundecane(ene) derivatives, we compared the effect of the two most inhibitory spiroundecane (**2** and **1**) and of one spiroundecene (**16**) derivative on the three reactions catalyzed by IN (3′-P, ST, and disintegration). As shown in Figure 2 these compounds show similar inhibition for 3′-P (21 bp duplex as a substrate, Fig. 2A, Table 1) and ST (precleaved substrate), Figure 2B, IC_{50} are 10.3 ± 5.0 μM (compound **2**), 13.7 ± 4.4 μM (compound **1**), and 35.7 ± 16.3 μM (compound **16**). Therefore, spiroundecane(ene) derivatives are dual inhibitors of IN-mediated 3′P and ST. Spiroundecane(ene) derivatives also inhibit disintegration, which corresponds to the reverse reaction of strand transfer,[3] with comparable potency as for 3′-P

or ST for undecane derivatives (Fig. 2C), IC_{50} are 19.2 ± 1.2 μM (compound **2**), 81.4 ± 10.8 μM (compound **1**), and 213.0 ± 11.2 μM (compound **16**).

To explore whether spiroundecane(ene) derivatives' inhibitory properties were based on their ability to prevent DNA binding to IN, we investigated the effects of compound **1** on IN–DNA binding using two crosslinking strategies. First, we evaluated the ability of compound **1** to inhibit a crosslinking reaction between the cytosine in the 5′-AC overhang of the viral DNA and glutamine 148 of IN (Fig. 3A). A Q148C mutant form of HIV-1 IN allows specific covalent interaction with a thiol-modified cytosine in the 5′-AC overhang.[15] Figure 3 shows minimal interference with this specific IN–DNA contact. Marginal inhibition was only observed at the highest concentration (333 μM).

To further determine whether compound **1** could interfere with the IN–DNA binding at other sites, we used the Schiff-base assay.[16] This assay measures crosslinking between IN and a DNA substrate mimicking the viral U5 LTR end containing a single abasic site. We examined the effects of compound **1** on IN crosslinking at three different positions in DNA substrates (Fig. 3C). Compound **1** only marginally blocked the Schiff-base IN–DNA interactions (Fig. 3D), which is consistent with the results of the disulfide crosslinking assay. Together, the crosslinking results indicate that the spiroundecane derivative (compound **1**) has no significant

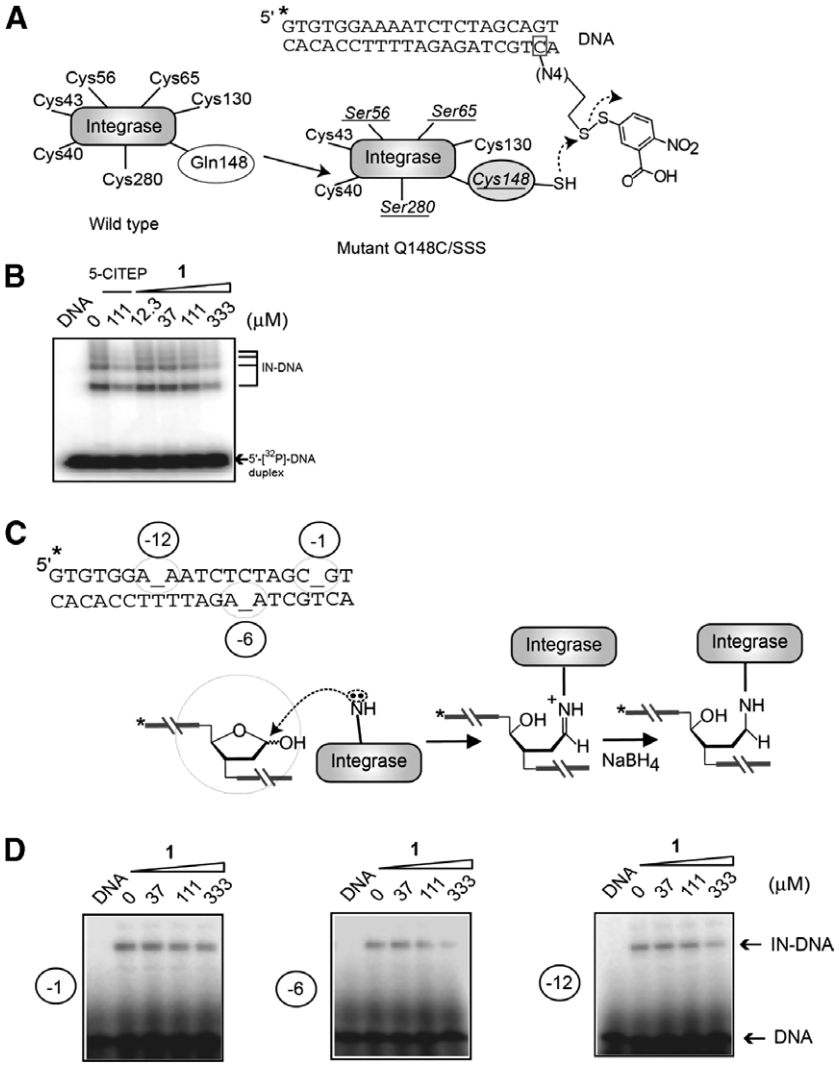

Figure 3. Compound **1** does not interfere with the formation of IN–DNA complexes at concentrations that inhibit IN catalytic activity. (A) IN–DNA disulfide crosslinking strategy. Left, schematic representation of the mutant IN used for crosslinking; upper right, modified oligonucleotide used for crosslinking[15] with a thioalkyl modification. The crosslinked complex forms between the cysteine residue 148 and the 5′-C from the DNA substrate (right). (B) SDS–PAGE analysis testing compound **1** in the disulfide crosslinking assay using the DNA substrate labeled with [^{32}P] at the 5′-end of the top strand. 5-CITEP (5-chloroindolyltetrazolylpropenone) was used as a positive control for crosslinking inhibition.[15] (C) Principle of the Schiff-base crosslinking assay. An abasic site is introduced by uracil DNA glycosylase in the DNA substrate containing uracil at the −1 (corresponding to the adenine in the conserved CA-dinucleotide), −12 or −6 positions. A nucleophile residue from IN (probably lysine) attacks the C1′-carbon of the abasic site.[16] Rearrangement of the initial enzyme–DNA complex leads to the formation of a Schiff-base intermediate stabilized with NaBH4. (D) SDS–PAGE analysis showing the testing of compound **1** on the crosslinking reactions between IN and DNA. The asterisks indicate the 5′-[^{32}P]-label.

interference with IN–DNA binding at concentrations that block IN catalytic activity.

None of the compounds that inhibit HIV integrase displayed cytoprotective activity for MT-2 cells infected by HIV_{IIIB}, but all of them had moderate toxicity in this type of cells ($CC_{50} > 111$ μM). Probably, low cell penetration ability leads to failure of antiviral activity. Therefore, the design of analogues of this novel chemotype will be necessary for antiviral activity. Such work is currently in progress and will be reported elsewhere.

In conclusion, we have synthesized and evaluated a series of original spiroundecane(ene) derivatives as HIV-1 integrase inhibitors. Spiroundecane(ene) derivatives are dual inhibitors of both reactions (3′-processing and strand transfer) catalyzed by IN. An undecane core with at least one furan moiety is preferred for IN inhibition. Structure–activity comparison provides evidence that the presence of an oxygen-containing substitution in the benzene is important for inhibition of IN. Crosslinking data suggest that spiroundecane derivatives interfere with the IN catalytic activity without significantly affecting IN–DNA binding. The moderate toxicity of spiroundecane(ene) derivatives encourages the further design of therapeutically relevant analogues based on of this novel chemotype of IN inhibitors.

Acknowledgments

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8. General procedure for the synthesis of the 2,4-dioxaspiro[5,5]undecane-1,5,9-triones **1**–**11**. A solution of 3-trimethylsiloxy-1,3-butadiene **12** (1.14 g, 5.5 mmol) in 5 ml acetonitrile was added under stirring to a suspension of dienophile (5 mmol) and 0.03 g L-proline in 50 ml acetonitrile. The mixture was stirred at rt for 25–40 h. Upon evaporation of solvent, a residue was treated with 10 ml of 3% NH_4OH. The mixture was stirred for 10 min and then treated with 50 ml methylene chloride. The organic layer was separated, while the water one was extracted with methylene chloride. The collected extracts are washed (with water and brine) and dried ($MgSO_4$). The solvent was evaporated and the residue was purified by column chromatographies. The eluates, containing the products, are evaporated, and residues are recrystallized from the corresponding solvent.
3,3-Dimethyl-7,11-di-(furan-2-yl)-2,4-dioxaspiro[5,5]undecane-1,5,9-trione **(1)**. Yield 92%, mp 198–202 °C (from acetonitrile), ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 1.02 (6H, s, 2×CH_3–C^3), 2.59 (2H, dd, J = 16.5, 4.5 Hz, H-8,10), 3.52 (2H, m, H_1-8,10), 4.07 (2H, dd, J = 14.1, 4.7 Hz, H-7,11), 6.20 (1H, d, J = 3.5 Hz, H_1-3′), 6.30 (1H, dd, J = 3.5, 2.1 Hz, H-4′), 7.33 (1H, dd, J = 2.1, 0.8 Hz, H_1-5′). ^{13}C NMR ($CDCl_3$, 300 MHz) δ_C: 24.8 (2xCH_3), 41.6 ($C^{8,10}$), 43.8 ($C^{7,11}$), 57.7 (C^6), 106.3 (C^3), 109.6 ($C^{3',3''}$), 111.7 ($C^{4',4''}$), 143.9 ($C^{5',5''}$), 152.4 ($C^{2',2''}$), 165.2 (C^1), 168.5 (C^5), 206.4 (C^9). IR (cm^{-1}): 898, 1500, 1589, 1636 (C=C), 1285, 1760 (C=I). Anal. Calcd for $C_{19}H_{18}O_7$: C, 63.69; H, 5.03. Found: C, 63.4; H, 5.1. 11-(1*H*-Indol-3-yl)-3,3-dimethyl-7-(furan-2-yl)-2,4-dioxa-spiro[5,5] undecane-1,5,9-trione **(2)**. Yield 65%, mp 167–169 °C (from ethanol–ether). ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 0.46 (3H, s, CH_3–C^3), 0.96 (3H, s, CH_3–C^3), 2.73 (2H, m, H-8,10), 3.71 (2H, m, H_1-8,10), 4.19 (1H, dd, J = 14.0, 4.8 Hz, H-7), 4.32 (1H, dd, J = 14.2, 4.6 Hz, H-11), 6.20 (1H, d, J = 3.3 Hz, H_1-3′), 6.30 (1H, dd, J = 3.3, 1.8 Hz, H-4′), 6.84 (1H, d, J = 1.5 Hz, H-2″), 7.01 (1H, dt, J = 7.5, 7.5, 1.1 Hz, H-6″), 7.15 (2H, m, H-7″,5″), 7.30 (1H, dd, J = 1.8, 0.8 Hz, H_1-5′), 7.38 (1H, d, J = 7.8, H-4″), 8.9 (1H, s, NH). ^{13}C NMR ($CDCl_3$, 300 MHz) δ_C: 28.1 (CH_3), 28.5 (CH_3), 41.2 (C^{10}), 41.9 (C^8), 43.6 (C^7), 44.2 (C^{11}), 60.5 (C^6), 106.1 (C^3), 108.5 ($C^{3'}$), 110.7 ($C^{4'}$), 111.0 ($C^{7''}$), 113.6 ($C^{3''}$), 119.6, 119.7 ($C^{4'',5''}$), 122.7 ($C^{2''}$), 123.3 ($C^{6''}$), 125.4 ($C^{3a''}$), 135.5 ($C^{7a''}$), 142.5 ($C^{5'}$), 151.1 ($C^{2'}$), 167.0 (C^1), 168.7 (C^5), 206.8 (C^9). Anal. Calcd for $C_{23}H_{21}NO_6$: C, 67.81; H, 5.16; N, 3.44. Found: C, 67.7; H, 5.3; N, 3.7.
3,3-Dimethyl-11-phenyl-7-(furan-2-yl)-2,4-dioxa-spiro[5,5]-undecane-1,5,9-trione **(3)**. Yield 81%, mp 186–187 °C (from ethyl acetate). ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 0.56 (3H, s, CH_3–C^3), 0.96 (3H, s, CH_3–C^3), 2.60 (2H, m, H-8,10), 3.57 (2H, m, H_1-8,10), 3.85 (1H, dd, J = 13.6,

4.2 Hz, H-7), 4.09 (1H, dd, J = 13.5, 4.6 Hz, H-11), 6.17 (1H, d, J = 3.2 Hz, H_1-3′), 6.27 (1H, dd, J = 3.2, 2.8 Hz, H-4′), 7.15–7.35 (6H, m, H_5–Ph, H_1-5′). IR (cm^{-1}): 720, 900, 1497, 1589, 1610 (C═C), 1712, 1730, 1763 (C═I). Anal. Calcd for $C_{21}H_{20}O_6$: 368.12598. Found: 368.12505.

3,3-Dimethyl-11-(*p*-methoxyphenyl)-7-(furan-2-yl)-2,4-dioxa-spiro[5,5] undecane-1,5,9-trione **(4)**. Yield 71%, mp 165–167 °C (ethyl acetate). ^{1}H NMR($CDCl_3$, 400 MHz) δ: 0.67 (3H, s, CH_3–C^3), 0.98 (3H, s, CH_3–C^3), 2.59 (2H, m, H-8,10), 3.56 (2H, m, H_1-8,10), 3.73 (3H, s, CH_3I–$C^{4''}$), 3.85 (1H, dd, J = 13.4, 4.5 Hz, H-7), 4.11 (1H, dd, J = 13.5, 4.6 Hz, H-11), 6.17 (1H, d, J = 3.0 Hz, H_1-3′), 6.27 (1H, dd, J = 3.0, 2.6 Hz, H-4′), 6.82 (2H, d, J = 8.2 Hz, H-3″ and 5″), 7.11 (2H, d, J = 8.2 Hz, H-2″ and 6″), 7.31 (1H, d, J = 2.6 Hz, H-5′). ^{13}C NMR ($CDCl_3$, 300 MHz) δ_C: 28.4 (CH_3), 28.2 (CH_3), 41.1 (C^{10}), 42.8 (C^8), 43.6 (C^7), 48.6 (C^{11}), 55.1 (OCH_3), 60.8 (C^6), 106.1 (C^3), 108.5 ($C^{3'}$), 110.6 ($C^{4'}$), 114.3 ($C^{3'',5''}$), 128.8 ($C^{1''}$), 129.1 ($C^{2'',6''}$), 142.4 ($C^{5'}$), 150.9 ($C^{2'}$), 159.5 ($C^{4''}$), 164.7 (C^1), 168.3 (C^5), 206.4 (C^9). IR (cm^{-1}): 910, 1514, 1581, 1611 (C═C), 1724, 1759 (C═I). Anal. Calcd for $C_{22}H_{22}O_7$: 398.13654. Found: 398.13605.

11-(*p*-Fluorophenyl)-3,3-dimethyl-7-(furan-2-yl)-2,4-dioxa-spiro[5,5]-undecane-1,5,9-trione **(5)**. Yield 63%, mp 175–178 °C (from ethyl acetate). ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 0.70 (3H, s, CH_3–C^3), 0.97 (3H, s, CH_3–C^3), 2.61 (2H, m, H-8,10), 3.57 (2H, m, H_1-8,10), 3.90 (1H, dd, J = 13.4, 4.5 Hz, H-7), 4.13 (1H, dd, J = 13.3, 4.7 Hz, H-11), 6.19 (1H, d, J = 3.0 Hz, H_1-3′), 6.30 (1H, dd, J = 3.0, 2.8 Hz, H-4′), 7.01 (2H, d, J = 8.3 Hz, H-3″ and 5″), 7.19 (2H, d, J = 8.3 Hz, H-2″ and 6″), 7.33 (1H, d, J = 2.8 Hz, H-5′). ^{13}C NMR ($CDCl_3$, 300 MHz) δ_C: 28.3 (CH_3), 28.5 (CH_3), 41.3 (C^{10}), 42.8 (C^8), 43.8 (C^7), 48.8 (C^{11}), 58.7 (C^6), 106.3 (C^3), 108.8 ($C^{3'}$), 110.8 ($C^{4'}$), 115.9 ($C^{3'',5''}$), 130.1 ($C^{1''}$), 130.3 ($C^{2'',6''}$), 142.7 ($C^{5'}$), 150.7 ($C^{2'}$), 164.7 ($C^{4''}$), 165.1 (C^1), 168.0 (C^5), 205.9 (C^9). Calcd for $C_{21}H_{19}O_6F$: 386.11655. Found: 386.11578.

11-(*p*-Bromophenyl)-3,3-dimethyl-7-(furan-2-yl)-2,4-dioxa-spiro[5,5]undecane-1,5,9-trione **(6)**. Yield 81%, mp 145–147 °C (from ethyl acetate). ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 0.73 (3H, s, CH_3–C^3), 0.98 (3H, s, CH_3-C^3), 2.57 (1H, dd, J = 13.0, 4.4 Hz, H-10), 2.65 (1H, dd, J = 13.2, 4.0 Hz, H-8), 3.57 (2H, m, H_1-8,10), 3.87 (1H, dd, J = 13.6, 4.0 Hz, H-7), 4.13 (1H, dd, J = 13.3, 4.4 Hz, H-11), 6.20 (1H, d, J = 3.3 Hz, H_1-3′), 6.30 (1H, dd, J = 3.3, 2.8 Hz, H-4′), 7.09 (2H, d, J = 8.0 Hz, H-2″ and 6″), 7.46 (2H, d, J = 8.0 Hz, H-3″ and 5″), 7.33 (1H, d, J = 2.8 Hz, H-5′). ^{13}C NMR ($CDCl_3$, 300 MHz) δ_C: 28.3 (CH_3), 28.5 (CH_3), 41.2 (C^{10}), 42.4 (C^8), 43.7 (C^7), 48.9 (C^{11}), 58.4 (C^6), 106.4 (C^3), 108.9 ($C^{3'}$), 110.8 ($C^{4'}$), 130.0 ($C^{2'',6''}$), 122.8 ($C^{1''}$), 132.3 ($C^{3'',5''}$), 135.7 ($C^{4''}$), 142.6($C^{5'}$), 150.6 ($C^{2'}$), 164.6 (C^1), 167.9 (C^5), 205.9 (C^9). Anal. Calcd for $C_{21}H_{19}O_6Br$: 446.03654. Found: 446.03711.

11-(*o*-Chlorophenyl)-3,3-dimethyl-7-(furan-2-yl)-2,4-dioxa-spiro[5,5]undecane-1,5,9-trione **(7)**. Yield 71%, mp 174–177 °C (from ethyl acetate). ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 0.89 (3H, s, CH_3–C^3), 0.95 (3H, s, CH_3–C^3), 2.55 (1H, dd, J = 15.0, 4.4 Hz, H-10), 2.67 (1H, dd, J = 15.2, 4.6 Hz, H-8), 3.38 (1H, m, H_1-8), 3.65 (1H, m, H_1-10), 4.23 (1H, dd, J = 13.5, 4.6 Hz, H-7), 4.69 (1H, dd, J = 14.0, 4.4 Hz, H-11), 6.21 (1H, d, J = 3.1 Hz, H_1-3′), 6.29 (1H, dd, J = 3.1, 2.0 Hz, H-4′), 7.20–7.41 (6H, m, H_4–Ph, H_1-5′). ^{13}C NMR ($CDCl_3$, 300 MHz) δ_C: 28.0 (CH_3), 28.9 (CH_3), 41.4 (C^{10}), 43.3 (C^8), 43.6 (C^7), 44.6 (C^{11}), 56.7 (C^6), 106.3 (C^3), 109.2 ($C^{3'}$), 110.8 ($C^{4'}$), 127.3 ($C^{3''}$), 128.4 ($C^{5''}$), 129.3 ($C^{1''}$), 130.6 ($C^{4''}$), 134.2 ($C^{6''}$), 134.7 ($C^{2''}$), 142.5 ($C^{5'}$), 150.8 ($C^{2'}$), 165.2 (C^1), 166.8 (C^5), 205.8 (C^9). Anal. Calcd for $C_{21}H_{19}ClO_6$. C, 62.61; H, 4.72; Cl, 8.81. Found: C, 62.8; H, 4.9; Cl, 9.1.

11-(3-Hydroxy-4-methoxyphenyl)-3,3-dimethyl-7-(furan-2-yl)-2,4-dioxa-spiro[5,5] undecane-1,5,9-trione **(8)**. Yield 83%, mp 197–198 °C (from acetonitrile). ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 0.73 (3H, s, CH_3–C^3), 1.01 (3H, s, CH_3–C^3), 2.57 (1H, ddd, J = 15.5, 4.4, 1.0 Hz, H-10), 2.53 (1H, ddd, J = 15.2, 4.6, 1.2 Hz, H-8), 3.55 (2H, m, H_1-8,10),3.80 (1H, dd, J = 13.9, 4.4 Hz, H-7), 3.83 (3H, s, CH_3I–$C^{4''}$), 4.10 (1H, dd, J = 14.2, 4.7 Hz, H-11), 6.18 (1H, d, J = 3.3 Hz, H_1-3′), 6.29 (1H, dd, J = 3.3, 2.0 Hz, H-4′), 6.68 (1H, dd, J = 8.4, 2.3 Hz, H-6″), 6.80 (1H, d, J = 2.3, H-2″), 6.98 (1H, d, J = 8.4, H-5″), 7.32 (1H, dd, J = 2.0, 0.9 Hz, H-5′), 10.8 (1H, s, OH). ^{13}C NMR ($CDCl_3$, 300 MHz) δ_C: 28.3 (CH_3), 28.5 (CH_3), 41.3 (C^{10}), 43.0 (C^8), 44.0 (C^7), 48.8 (C^{11}), 56.0 (OCH_3), 58.6 (C^6), 106.2 (C^3), 108.6 ($C^{3'}$), 110.8, 110.9 ($C^{4',6''}$), 114.5 ($C^{5''}$), 120.2 ($C^{2''}$), 130.1 ($C^{1''}$), 142.4 ($C^{2'}$), 146.1, 146.8 ($C^{3'',4''}$), 151.0 ($C^{2'}$), 165.8 (C^1), 168.1 (C^5), 206.5 (C^9). Calcd for $C_{22}H_{22}O_8$: 414.13145. Found: 414.13134.

11-(3,4-Dimethoxy-phenyl)-3,3-dimethyl-7-(furan-2-yl)-2,4-dioxa-spiro[5,5] undecane-1,5,9-trione **(9)**. Yield 90%, mp 145–146 °C (from ether). ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 0.70 (3H, s, CH_3–C^3), 0.99 (3H, s, CH_3–C^3), 2.61 (2H, m, H-8,10), 3.56 (2H, m, H_1-8,10), 3.80 (1H, dd, J = 13.8, 4.5 Hz, H-7), 3.81 (6H, s, CH_3I–$C^{3'',4''}$), 4.11 (1H, dd, J = 15.0, 4.7 Hz, H-11), 6.18 (1H, d, J = 3.4 Hz, H_1-3′), 6.29 (1H, dd, J = 3.4, 2.0 Hz, H-4′), 6.68 (1H, dd, J = 8.2, 2.0 Hz, H-6″), 6.88 (1H, d, J = 2.0, H-2″), 6.92 (1H, d, J = 8.2, H-5″), 7.32 (1H, dd, J = 2.0, 0.9 Hz, H-5′). ^{13}C NMR ($CDCl_3$, 300 MHz)δ_C: 28.4 (CH_3), 28.5 (CH_3), 41.2 (C^{10}), 42.9 (C^8), 43.9 (C^7), 49.0 (C^{11}), 55.8, 55.9 (OCH_3), 58.7 (C^6), 106.2 (C^3), 108.6 ($C^{3'}$), 110.7, 111.3, 111.5 ($C^{4',5'',6''}$), 120.2 ($C^{2''}$), 129.2 ($C^{1''}$), 142.5 ($C^{2'}$), 149.1, 149.3 ($C^{3'',4''}$), 150.8 ($C^{2'}$), 165.5 (C^1), 168.3 (C^5), 206.4 (C^9). Anal. Calcd for $C_{23}H_{24}O_8$: 428.14710. Found: 428.14761.

3,3-Dimethyl-11-(2-methyl-4-methoxy-phenyl)-7-(furan-2-yl)-2,4-dioxa-spiro[5,5]undecane-1,5,9-trione **(10)**. Yield 78%, mp 172–175 °C (from ether). ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 0.86 (3H, s, CH_3–C^3), 1.00 (3H, s, CH_3–C^3), 2.27 (3H, s, CH_3–$C^{2''}$), 2.27 (1H, dd, J = 15.2, 4.4 Hz, H-10), 2.65 (1H, dd, J = 16.0, 4.0 Hz, H-8), 3.44 (1H, m, H-8), 3.63 (1H, m, H-10), 3.72 (3H, s, CH_3I–$C^{4''}$), 4.22 (1H, dd, J = 14.8, 4.0 Hz, H-7), 4.31 (1H, dd, J = 15.0, 4.4 Hz, H-11), 6.19 (1H, d, J = 3.2 Hz, H_1-3′), 6.28 (1H, dd, J = 3.2, 2.0 Hz, H-4′), 6.67 (1H, d, J = 2.2 Hz, H-3″), 6.70 (1H, dd, J = 8.0, 2.2, H-5″), 7.18 (1H, d, J = 8.0, H-6″), 7.32 (1H, dd, J = 2.0, 0.8 Hz, H-5′). ^{13}C NMR ($CDCl_3$, 300 MHz) δ_C: 19.6 (CH_3), 28.2 (CH_3), 28.8 (CH_3), 41.4 (C^{10}), 43.9 (C^8), 44.0 (C^7), 44.5 (C^{11}), 55.1 (OCH_3), 57.3 (C^6), 106.4 (C^3), 108.8 ($C^{3'}$), 110.7 ($C^{4'}$), 112.2 ($C^{5''}$),116.3 (6″), 127.8 ($C^{1''}$), 128.1 ($C^{3''}$), 138.3 ($C^{2''}$), 142.5 ($C^{2'}$), 151.0 ($C^{2'}$), 158.9 ($C^{4''}$),165.4 (C^1), 168.0 (C^5), 206.7 (C^9). Anal. Calcd for $C_{23}H_{24}O_7$: 412.15219. Found: 412.15340.

11-(2,3-Dimethoxy-phenyl)-3,3-dimethyl-7-(furan-2-yl)-2,4-dioxa-spiro[5,5] undecane-1,5,9-trione **(11)**. Yield 74%, mp 127–128 °C (from ethyl acetate). ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 0.85 (3H, s, CH_3–C^3), 0.92 (3H, s, CH_3–C^3), 2.51 (2H, m, H-8,10), 3.57 (2H, m, H_1-8,10), 3.76 (6H, s, CH_3I–$C^{2'',3''}$), 4.17 (1H, dd, J = 14.6, 4.2 Hz, H-7), 4.43 (1H, dd, J = 14.5, 4.5 Hz, H-11), 6.13 (1H, d, J = 3.0 Hz, H_1-3′), 6.22 (1H, dd, J = 3.0, 2.0 Hz, H-4′), 6.76 (2H, d, J = 8.4 Hz, H-4″,6″), 6.92 (1H, m, H-5″), 7.27 (1H, dd, J = 2.0, 0.9 Hz, H-5′). ^{13}C NMR ($CDCl_3$, 300 MHz) δ_C: 27.9 (CH_3), 29.0 (CH_3), 41.1 (C^{10}), 41.4 (C^8), 43.2 (C^7), 43.3 (C^{11}), 55.6, 55.7 (OCH_3), 57.1 (C^6), 106.1 (C^3), 108.8 ($C^{3'}$), 108.9, 110.5, 110.7 ($C^{4',5'',6''}$), 112.3 ($C^{4''}$), 130.3 ($C^{1''}$), 142.5 ($C^{2'}$), 151.2 ($C^{2'}$), 152.7, 152.8 ($C^{2'',3''}$), 165.5 (C^1), 167.3 (C^5), 206.7 (C^9). Calcd for $C_{23}H_{24}O_8$: 428.14770. Found: 428.14761.

11-(3-Hydroxy-4-methoxyphenyl)-3,3-dimethyl-7-(2-methoxy-phenyl)-2,4-dioxa-spiro[5,5]undecane-1,5,9-trione **(17)** was obtained by the reaction of the diene **20** with 5-[1-(3-hydroxy-4-methoxy-phenyl)-ethylidene]-2,2-dimethyl-[1,3]-dioxane-4,6-dione **13h** by the above method. Yield 73%, mp 192–194 °C (from ethyl acetate). ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 0.70 (3H, s, CH_3–C^3), 1.05 (3H, s, CH_3–C^3), 2.59 (2H, m, H-8,10), 3.59 (2H, m, H_1-8,10), 3.77, 3.80 (6H, s, 2xCH_3I–$C^{2',4''}$), 4.08 (2H, m, H-7,11), 6.70 (1H, dd, J = 8.4, 2.3 Hz, H-6″), 6.82 (2H, m, J = 2.3, H-3′,2″), 6.98 (2H, H-5″,6′), 7.23 (2H, m, H-4′,5′), 10.8 (1H, s, OH). Anal. Calcd for $C_{25}H_{26}O_8$. C, 66.08; H, 5.69. Found: C, 66.5; H, 5.9.

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10. General procedure for the synthesis of the 2,4-dioxa-spiro[5,5]undec-8-ene-1,5-diones **16**, **19**: To a solution of Meldrum's acid **14** (0.46 g, 3.2 mmol), formaldehyde (0.5 ml of 37% equiv solution), and L-proline (0.03 g) in acetonitrile (10 ml), diene **15** (1.12 g, 4.0 mmol) (or **22**) was added at ambient temperature in a period of 10 min. The reaction mixture was stirred for 1.5 h, quenched with ice-water, the product extracted by methylene chloride, the combined organic layer was dried ($MgSO_4$), evaporated in vacuum, and the residue was purified by chromatography on silica gel. The residues are recrystallized from the corresponding solvent.
9-Hydroxy-3,3-dimethyl-7-(furan-2-yl)-1,5-dioxo-2,4-dioxa-spiro[5,5]undec-8-ene-8-carboxylic acid ethyl ester **(16)**. Yield 77%, mp 125–128 °C (from ethyl acetate). IR (cm^{-1}): 710, 750, 840, 1505, 1625 (C═C), 1720, 1760 (C═O), 3090, 3420 (OH). 1H NMR ($CDCl_3$, 400 MHz) δ: 0.98 (3H, t, J = 7.0 Hz, CH_3), 1.63 (3H, s, CH_3–C^3), 1.68 s (3H, s, CH_3–C^3), 2.20 (1H, m, H_1-10), 2.41 (1H, m, H_1-11), 2.78 (2H, m, H-10,11), 4.07, 4.11 (2H, dd, J = 7.0 Hz, CH_2), 4.68 (1H, s, H-7), 6.04 (1H, dd, J = 4.6, 1.6 Hz, H-3′) 6.25 (1H, dd, J = 4.6, 3.0, H-4′), 7.26 (1H, dd, J = 3.0, 1.0, H-5′), 12.4 s (1H, OH). Anal. Calcd for $C_{18}H_{20}O_8$: C, 59.30; H, 5.49. Found: C, 59.5; H, 5.8.
9-Hydroxy-3,3-dimethyl-7-(2-methoxyphenyl)-1,5-dioxo-2,4-dioxa-spiro[5,5] undec-8-ene-8-carboxylic acid ethyl ester **(19)**. Yield 86%, mp 128–130 °C (from ethyl acetate). IR (cm^{-1}): 720, 750, 820, 880, 1040, 1080, 1120, 1260, 1360, 1500, 1560, 1580, 1620, 1710, 1720, 1740, 1780. 1H NMR ($CDCl_3$, 400 MHz) δ: 0.84 (3H, t, J = 7.0 Hz, CH_3), 1.64 (3H, s, CH_3–C^3), 1.67 (3H, s, CH_3–C^3), 2.14 (1H, m, H-5), 2.30 (1H, m, H-6), 2.72 (2H, m, H-5,6), 3.76 s (3H, OCH_3), 3.91 (2H, q, J = 7.0 Hz, CH_2), 4.99 (1H, s, H-7), 6.79 (1H, dd, J = 7.6, 1.0, H-3′), 6.85 (1H, dd, J = 7.2, 1.0, H-5′), 6.99 (1H, dd, J = 7.6, 1.8, H-6′), 7.18 (1H, dd, J = 7.2, 1.8, H-4′), 12.4 s (1H, OH). ^{13}C NMR ($CDCl_3$, 300 MHz) δ_C: 13.9 (CH_3), 25.8, 26.1 ($C^{10,11}$), 27.7 (CH_3), 29.8 (CH_3), 51.4 (C^7), 54.8 (C^6), 55.4 (ICH_2), 60.1 (CH_2), 104.8 (C^3), 109.9 ($C^{3'}$), 120.6($C^{6'}$), 127.4 ($C^{1'}$), 128.5 ($C^{4'}$), 129.2 ($C^{5'}$), 130.9 (C^8), 156.9 ($C^{2'}$), 162.9 (C^9), 166.0 (C^1), 168.2 (C^5), 172.7 (C═O). Anal. Calcd for $C_{21}H_{24}O_8$: C, 62.38; H, 6.0. Found: C, 62.2; H, 5.8.
3,3-Dimethyl-7-(4-methoxyphenyl)-2,4-dioxa-spiro[5,5]undecane-1,5,9-trione **(18)**. To a solution of Meldrum's acid **14** (0.46 g, 3.2 mmol), formaldehyde (0.5 ml of 37% equiv solution), and L-proline (0.03 g) in acetonitrile (10 ml), diene **21** (1.01 g, 4.0 mmol) was added at ambient temperature in a period of 10 min. The reaction mixture was stirred for 1.5 h, quenched with ice-water, the product extracted by methylene chloride, the combined organic layer was dried ($MgSO_4$) and evaporated in vacuum. Purification by silica gel chromatography eluting with 1% ethanol in chloroform yielded 0.82 g (62%) of the desired product. Mp 112–114 °C. ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 1.58 (3H, s, CH_3–C^3), 1.72 (3H, s, CH_3–C^3), 2.38 (2H, m, H-10,11), 2.69 (1H, m, H-8), 3.30 (1H, dd, J 12.3, 5.0, H-7), 3.62 (2H, m, H-10,11), 3.78 (3H, s, ICH_3–$C^{4'}$), 4.26 (1H, dd, J 13.8, 5.0 H-8), 6.75 (1H, dd, J = 7.6, 1.0 Hz, H-3′), 6.88 (1H, dd, J 7.2, 1.0 Hz, H-5′), 7.08 (1H, dd, J = 7.2, 1.8 Hz, H-6′), 7.14 (1H, d, J = 7.6 Hz, H-2′). Anal. Calcd for $C_{18}H_{20}O_6$: C, 65.06; H, 6.02. Found: C, 65.3; H, 6.1.
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12. X-ray crystallographic analysis for compound **1**: the data were collected on a Bruker P4 diffractometer with Mo K_α radiation. The cell parameters and the orientation matrix for data collection were obtained from least-squares refinement in the range of 20–25 °C (2θ). For measurement a single crystal of **1** $C_{19}H_{18}O_7$ [0.68 mm × 0.25 mm × 0.06 mm] was chosen. Monoclinic system with the following unit cell parameters: a = 9.362(2), b = 9.404(2), c = 19.889(3) Å, β = 92.48(1)°, V = 1749.4(6) Å^3, space group $P2_1/n$, Z = 4, d_{calc} = 1.361 Γ/cm^3, μ = 0.105 mm^{-1}. A total of 3268 unique reflections were measured (among them 3066 independent R(int) = 0.0260). A correction for absorption was introduced empirically by psi-curves (transmission 0.84–0.93). The structure was solved by the direct method with the use of SHELXS-97 software. The refining of structural parameters was carried out by the least-squares procedure in the full-matrix anisotropic approximation applying the program SHELXL-97. Hydrogen atoms were included in the refinement restrained to ride on the atom to which they are bonded. The final refinement of the structure parameters was performed with respect to all F^2 to wR_2 = 0.1855, S = 1.010; 236 parameters (R = 0.0652 for 1535 $F > 4\sigma$). The crystal structure of compound **1** has been deposited at the Cambridge Crystallographic Data Centre and allocated the deposition number CCDC 601465. Data Acquisition—the Cambridge crystallographic Data Centre deposit@ccdc.cam.ac.uk; http://www.ccdc.cam.ac.uk/deposit; Telephone: 44 01223 762910; Facsimile: 44 01223 336033; Address: CCDC, 12 Union Road, CAMBRIDGE CB2,1EZ, UK.
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