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Synthesis and Biological Investigation of Δ^{12}-Prostaglandin J_3 (Δ^{12}-PGJ_3) Analogues and Related Compounds

K. C. Nicolaou,*[,†] Kiran Kumar Pulukuri,[†,§] Stephan Rigol,[†,§] Philipp Heretsch,[†,§] Ruocheng Yu,[†] Charles I. Grove,[†] Christopher R. H. Hale,[†] Abdelatif ElMarrouni,[†] Verena Fetz,[‡] Mark Brönstrup,[‡] Monette Aujay,[#] Joseph Sandoval,[#] and Julia Gavrilyuk[#]

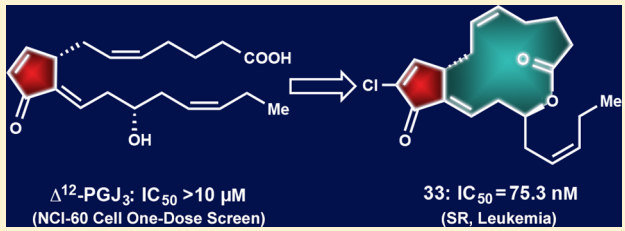
[†]Department of Chemistry, BioScience Research Collaborative, Rice University, 6100 Main Street, Houston, Texas 77005, United States

[‡]Department of Chemical Biology, Helmholtz Centre for Infection Research, Inhoffenstraße 7, 38124 Braunschweig, Germany

[#]Stemcentrx Inc., 450 East Jamie Court, South San Francisco, California 94080, United States

Supporting Information

ABSTRACT: A series of Δ^{12}-prostaglandin J_3 (Δ^{12}-PGJ_3) analogues and derivatives were synthesized employing an array of synthetic strategies developed specifically to render them readily available for biological investigations. The synthesized compounds were evaluated for their cytotoxicity against a number of cancer cell lines, revealing nanomolar potencies for a number of them against certain cancer cell lines. Four analogues (**2**, **11**, **21**, and **27**) demonstrated inhibition of nuclear export through a covalent addition at Cys528 of the export receptor Crm1. One of these compounds (i.e., **11**) is currently under evaluation as a potential drug candidate for the treatment of certain types of cancer. These studies culminated in useful and path-pointing structure−activity relationships (SARs) that provide guidance for further improvements in the biological/pharmacological profiles of compounds within this class.



1. INTRODUCTION

As described in a separate article[1] and a previous communication,[2] we developed a number of synthetic strategies for the synthesis of the reported antileukemic agent Δ^{12}-prostaglandin J_3 (Δ^{12}-PGJ_3, **1**, Figure 1).[3,4] In this article, we describe the application of these synthetic technologies for the synthesis of a series of designed analogues and derivatives of this newly discovered prostanoid whose biological profile[3,4] elevated it to a lead compound for drug discovery. We also describe the results of a number of biological investigations with these compounds, including their evaluation as cytotoxic agents against a series of cancer cell lines, as well as aspects of their mechanism of action. Our motivation for the design and synthesis of the targeted molecules (**2**−**44**, *ent*-**1**, *ent*-**2**, *ent*-**11**, Figure 1) was to improve the stability and potency of the parent compound (Δ^{12}-PGJ_3, **1**) and to discover new molecular entities for further development as potential drug candidates for cancer chemotherapy.

2. RESULTS AND DISCUSSION

2.1. Synthesis of Δ^{12}-PGJ_3 Methyl Ester 2 and Lactone 11. In an effort to improve membrane permeability and stability, we synthesized the methyl ester (**2**) and lactone (**11**) derivatives of Δ^{12}-PGJ_3 through the use of $TMSCHN_2$ (93% yield) and MNBA/DMAP (Shiina method,[6] 71% yield), respectively, as summarized in Scheme 1. As will be discussed below, both the chemical stability and potency of derivatives **2** and **11** were improved over those of Δ^{12}-PGJ_3 (**1**), providing motivation for further molecular designs along these structural types.

2.2. Synthesis of Δ^{12}-PGJ_3 Ester (3−5 and 10), Amide Analogues (6−9) and Hydroxy Derivative 12. Ester derivatives of Δ^{12}-PGJ_3 (i.e., **3**−**5** and **10**, Scheme 2) were synthesized through their TBS−ether precursors (**46**−**49**, respectively) prepared from TBS−ether **45**[2] by EDCI/DMAP-facilitated esterification as summarized in Scheme 2. The latter were desilylated by exposure to aq HF as shown in Scheme 2. In addition to the esters and in a similar manner, we synthesized a series of amide analogues (**6**−**9**, Scheme 3) from TBS−ether **45**[2] by standard methods (Boc_2O/NH_4HCO_3 or EDCI/HOBt) via their TBS−ether derivatives (**50**−**53**, respectively, Scheme 3). The C1-hydroxy analogue **12** was also prepared from OPMB derivative **54**[2] through desilylation (aq HF) and deprotection (DDQ) via intermediate **55** as shown in Scheme 4.

2.3. Synthesis of *ent*-Δ^{12}-PGJ_3 Analogues *ent*-1, *ent*-2, and *ent*-11. To test the importance of the absolute configuration of Δ^{12}-PGJ_3 (**1**) to its biological activity, we set out to prepare its enantiomer (*ent*-Δ^{12}-PGJ_3, *ent*-**1**) and its derivatives, methyl ester (*ent*-**2**) and lactone (*ent*-**11**). As seen in Scheme 5, we took advantage of our menthol-chiral approach[1] to this structural type to generate the required enantiomeric enone *ent*-**57** (generated from **56**). *ent*-**57** was converted, through an

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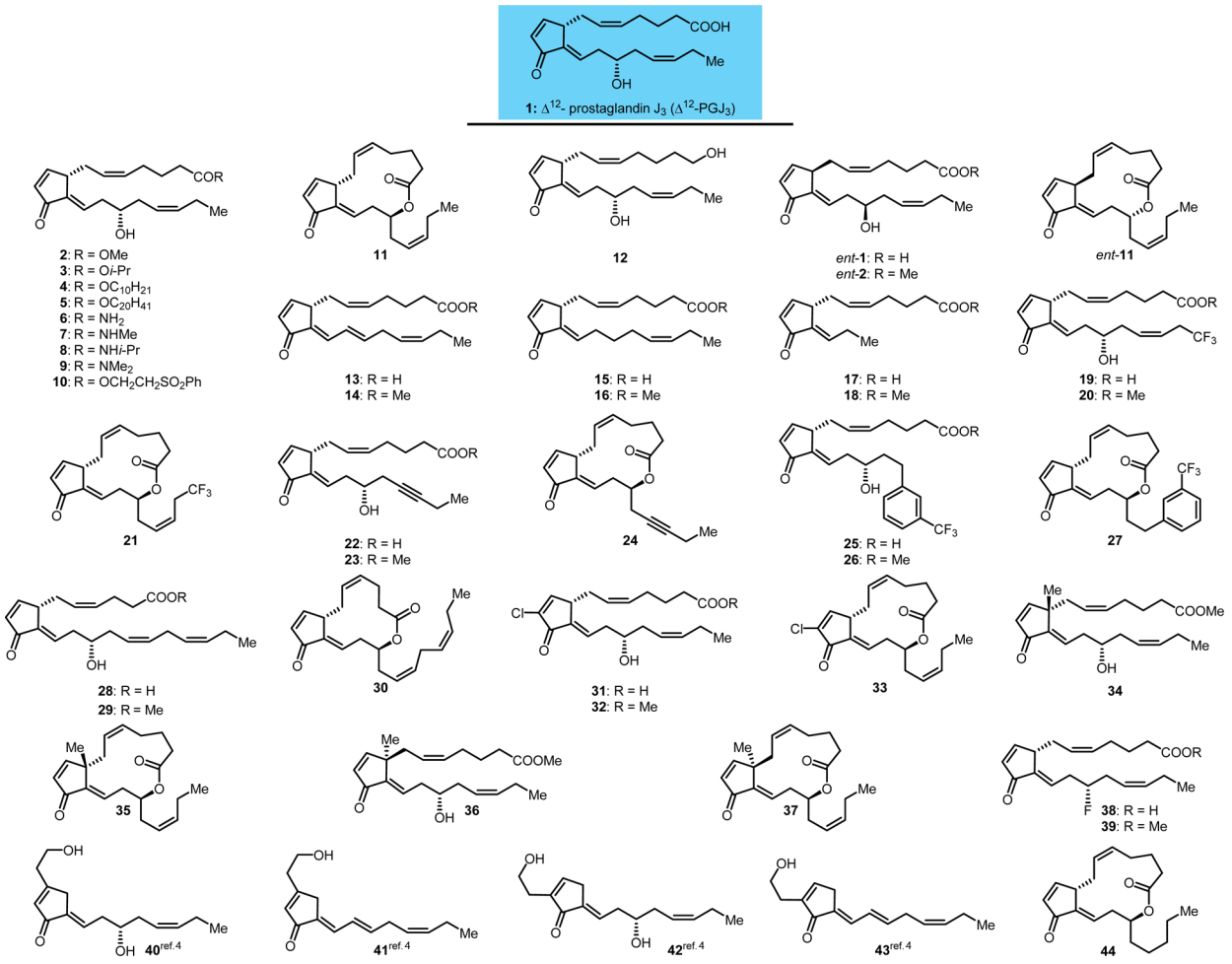
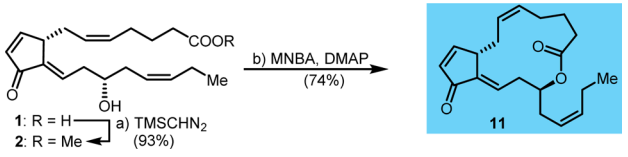


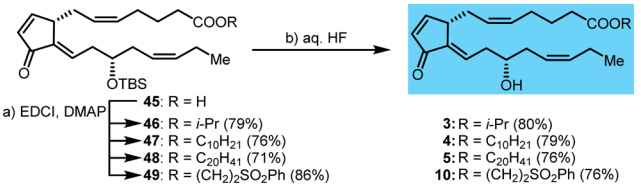
Figure 1. Molecular structures of Δ^{12}-prostaglandin J_3 (Δ^{12}-PGJ_3, **1**) and designed analogues **2**–**44**, *ent*-**1**, *ent*-**2**, and *ent*-**11**.

Scheme 1. Synthesis of Δ^{12}-PGJ_3 Methyl Ester 2 and Lactone 11[a]



[a]Reagents and conditions: (a) $TMSCHN_2$ (2 M in Et_2O, 1.5 equiv), 3:2 C_6H_6/MeOH, 25 °C, 30 min, 93%; (b) MNBA (1.4 equiv), DMAP (6.0 equiv), CH_2Cl_2, 25 °C, 17 h, 74%; MNBA = 2-methyl-6-nitrobenzoic anhydride, DMAP = 4-dimethylaminopyridine.

Scheme 2. Synthesis of Δ^{12}-PGJ_3 Ester Analogues 3–5 and 10[a]



[a]Reagents and conditions: (a) **46**: EDCI (2.0 equiv), DMAP (0.05 equiv), *i*-PrOH (1.5 equiv), CH_2Cl_2, 0 °C, 6 h, 79%; **47**: EDCI (2.0 equiv), DMAP (0.05 equiv), $C_{10}H_{21}OH$ (1.5 equiv), CH_2Cl_2, 0 to 25 °C, 12 h, 76%; **48**: EDCI (2.0 equiv), DMAP (0.05 equiv), $C_{20}H_{41}OH$ (1.5 equiv), CH_2Cl_2, 0 to 25 °C, 12 h, 71%; **49**: EDCI (1.5 equiv), DMAP (0.1 equiv), $PhSO_2(CH_2)_2OH$ (1.5 equiv), CH_2Cl_2, 0 °C, 6 h, 86%; (b) **3**: HF·NEt_3 (50 equiv), MeCN, 0 to 25 °C, 48 h, 80%; **4**: HF (50% aq, 100 equiv), MeCN, 0 °C, 30 min, 79%; **5**: HF (50% aq, 100 equiv), MeCN, 0 °C, 30 min, 76%; **10**: HF (50% aq, 50 equiv), MeCN, 0 °C, 30 min, 76%; EDCI = N-[3-(dimethylamino)propyl]-N′-ethylcarbodiimide.

aldol reaction with enantiomeric aldehyde *ent*-**58**,[5] to product **59** (mixture of diastereomers), which was transformed (MsCl, DMAP) to cross-dienone *ent*-**54** (single isomer) in 49% yield for the two steps. The rest of the synthesis of *ent*-**1**, *ent*-**2**, and *ent*-**11** proceeded through the same steps and in similar yields as described in a separate article for the synthesis of **1**[1] and summarized here in Scheme 5 (through intermediates **60**–*ent*-**45**).

2.4. Synthesis of Dehydrated $\Delta^{12,14}$-PGJ_3 Analogue 13 and Its Methyl Ester 14. To illuminate the effect of higher conjugation of the enone system of Δ^{12}-PGJ_3, and for comparison reasons, we prepared the dehydrated counterparts of the parent compounds **1** and **2**, namely 15-dehydroxy-$\Delta^{12,14}$-PGJ_3 (**13**) and its methyl ester analogue **14** from enone **57**[1] as shown in Scheme 6. Thus, intermediate **57** (obtained from the corresponding menthol-enone through DIBAL-H reduction[1]) was deprotected (DDQ, 94% yield), the resulting hydroxy enone (**62**) was silylated (TBSCl, 89% yield), and the resulting product (**63**) was coupled with conjugated aldehyde **64**[5] under the aldol conditions (LDA, −78 °C) to afford a mixture of alcohols (**65**). The latter was treated with MsCl in the presence of DMAP (0 to

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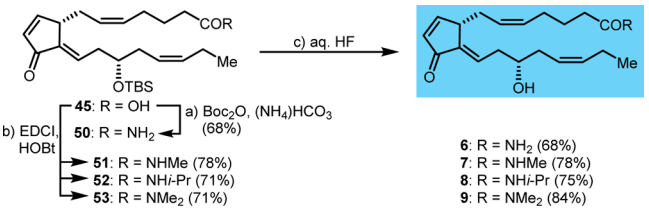
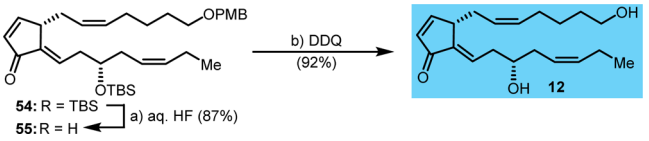
Scheme 3. Synthesis of Δ^{12}-PGJ$_3$ Amide Analogues 6–9[a]



[a]Reagents and conditions: (a) Boc_2O (2.6 equiv), $(NH_4)HCO_3$ (2.4 equiv), pyridine (1.2 equiv), CH_2Cl_2, 0 to 25 °C, 6 h, 68%; (b) **51**: EDCI (2.0 equiv), HOBt (2.0 equiv), H_2NMe (2.0 M in THF, 2.0 equiv), CH_2Cl_2, 0 to 25 °C, 2 h, 78%; **52**: EDCI (2.0 equiv), HOBt (2.0 equiv), H_2Ni-Pr (1.0 M in THF, 2.0 equiv), CH_2Cl_2, 0 to 25 °C, 2 h, 71%; **53**: EDCI (1.5 equiv), HOBt (1.5 equiv), $HNMe_2$ (1.0 M in THF, 1.2 equiv), CH_2Cl_2, 0 to 25 °C, 3 h, 71%; (c) **6**: HF (50% aq, 100 equiv), MeCN, 0 °C, 30 min, 68%; **7**: HF (50% aq, 100 equiv), MeCN, 0 °C, 30 min, 78%; **8**: HF (50% aq, 100 equiv), MeCN, 0 °C, 30 min, 75%; **9**: HF (50% aq, 100 equiv), MeCN, 0 °C, 30 min, 84%; Boc_2O = di-*tert*-butyl dicarbonate, HOBt = hydroxybenzotriazole.

Scheme 4. Synthesis of Δ^{12}-PGJ$_3$ Hydroxy Analogue 12[a]



[a]Reagents and conditions: (a) HF (50% aq, 50 equiv), MeCN, 0 °C, 2 h, 87%; (b) DDQ (1.5 equiv), 4:1 CH_2Cl_2/H_2O, 0 °C, 1 h, 92%; DDQ = 2,3-dichloro-5,6-dicyano-1,4-benzoquinone.

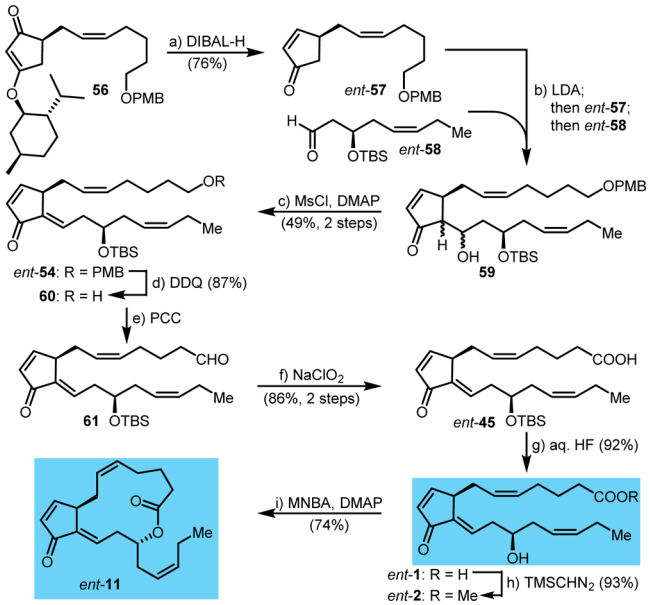
25 °C) to furnish conjugated enone **66** in 31% overall yield for the two steps. Desilylation of enone **66** (aq HF, 87% yield) was followed by stepwise oxidation (PCC, **68**; $NaClO_2$[7]) to furnish the targeted 15-deoxy-$\Delta^{12,14}$-PGJ$_3$ (**13**), from which methyl ester derivative **14** was generated by exposure to $TMSCHN_2$ (90% yield).

2.5. Synthesis of 15-Deoxy-Δ^{12}-PGJ$_3$ (15) and Its Methyl Ester 16. The 15-deoxy-Δ^{12}-PGJ$_3$ analogues **15** and **16** (Scheme 7) were synthesized in order to test the importance of the 15-hydroxy group of Δ^{12}-PGJ$_3$ for biological activity. To this end, and as shown in Scheme 7, enone **57**[1] was reacted with aldehyde fragment **69**[5] under our aldol conditions (LDA, −78 °C), and the resulting mixture of alcohols (**70**) was exposed to the action of MsCl and DMAP to afford enone PMB derivative **71** in 61% overall yield for the two steps. The latter was oxidized fully and in one step by treatment with oxo-piperidinium tetrafluoroborate salt **72**[8] to afford, in 45% yield, targeted analogue **15**. Treatment of the latter with $TMSCHN_2$ furnished methyl ester analogue **16** (90% yield).

2.6. Synthesis of Δ^{12}-PGJ$_3$ Analogues 17 and 18. To test the effect of "lower" side chain modifications, truncated Δ^{12}-PGJ$_3$ analogues **17** and **18** ($TMSCHN_2$) were synthesized from key building blocks, enone **57**[1] and propionaldehyde (**73**), via intermediates **74** and **75** as illustrated in Scheme 8.

2.7. Synthesis of Δ^{12}-PGJ$_3$ Analogue 19, Its Methyl Ester 20, and Lactone 21. C20-trifluoro Δ^{12}-PGJ$_3$ analogues **19**–**21** were prepared from starting materials **76**,[9] **77**,[10] and **57**[1] through intermediates **78**–**85** by standard methods as summarized in Scheme 9. The motivation for this modification was the well-known benefits of fluorine residues not only to pharmacological properties[11] but also to block metabolic oxidation at C20.

Scheme 5. Synthesis of *ent*-Δ^{12}-PGJ$_3$ Analogues *ent*-1, *ent*-2, and *ent*-11[a]



[a]Reagents and conditions: (a) DIBAL-H (1.5 equiv), CH_2Cl_2, −10 °C, 30 min, 76%; (b) LDA (2.0 equiv); then *ent*-**57** (1.0 equiv); then *ent*-**58** (1.2 equiv), THF, −78 °C, 30 min; (c) MsCl (2.0 equiv), DMAP (10 equiv), CH_2Cl_2, 0 to 25 °C, 6 h, 49% for two steps; (d) DDQ (1.5 equiv), 16:1 CH_2Cl_2/H_2O, 0 °C, 45 min, 87%; (e) PCC (2.0 equiv), CH_2Cl_2, 25 °C, 2 h; (f) $NaClO_2$ (3.0 equiv), NaH_2PO_4 (3.0 equiv), 2-methyl-2-butene (10 equiv), *t*-BuOH, 25 °C, 30 min, 86% for two steps; (g) HF (50% aq, 100 equiv), MeCN, 0 °C, 45 min, 92%; (h) $TMSCHN_2$ (2 M in Et_2O), 3:2 C_6H_6/MeOH, 25 °C, 30 min, 93%; (i) MNBA (1.4 equiv), DMAP (6.0 equiv), CH_2Cl_2, 25 °C, 17 h, 74%; DIBAL-H = diisobutylaluminum hydride, LDA = lithium diisopropylamide, MsCl = methanesulfonyl chloride, PCC = pyridinium chlorochromate.

2.8. Synthesis of Alkyne Δ^{12}-PGJ$_3$ Analogue 22, Its Methyl Ester 23, and Lactone 24. In a similar fashion, acetylenic Δ^{12}-PGJ$_3$ analogues **22** and **23** were synthesized from key building blocks **86**,[5] and **57**[1] through the standard sequence via intermediates **88**–**93** as presented in Scheme 10. The 1,15-lactone **24** was prepared from hydroxy acid **22** by exposure to MNBA and DMAP as shown in Scheme 10.

2.9. Synthesis of Δ^{12}-PGJ$_3$ Analogue 25, Its Methyl Ester 26, and Lactone 27. The synthesis of Δ^{12}-PGJ$_3$ analogues **25**–**27** carrying a trifluoromethyl phenyl residue at the end of the "lower" chain from building blocks **57**,[1] lactol **94**,[12] and phosphonium salt **95**[13] is shown in Scheme 11. Thus, Wittig reaction of the ylide derived from **95** with lactol **94** (*n*-BuLi) gave an inconsequential mixture of olefinic isomers **96** [(*E*)/(*Z*) = 65:35, 72% yield], reduction of which (10% Pd/C, H_2) led to primary alcohol **97** (91% yield). The latter was oxidized (DMP, 83% yield) to aldehyde **98**, which was coupled with enone **57** through an aldol reaction to afford coupling product **99** (mixture of diastereomers), whose elaboration to analogues **25**–**27** proceeded through the standard sequence, and intermediates **100**–**103** as summarized in Scheme 11.

2.10. Synthesis of Δ^{12}-PGJ$_3$ Analogue 28, Its Methyl Ester 29, and Lactone 30. Scheme 12 summarizes the synthesis of Δ^{12}-PGJ$_3$ analogues **28**–**30**. Inspired by docosahexaenoic acid (DHA) and a hypothesis for the plausible existence of **28** as a naturally existing substance, these analogues were chosen

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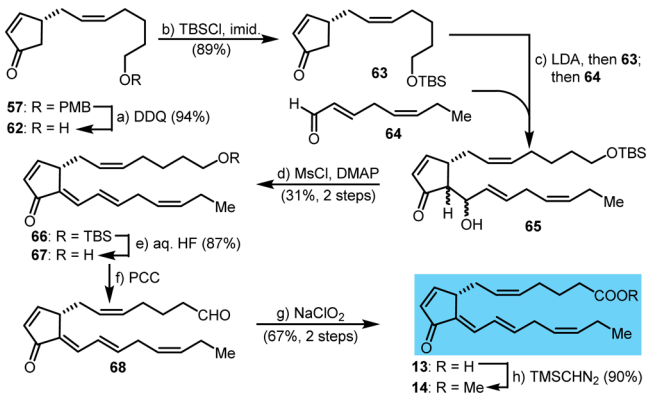
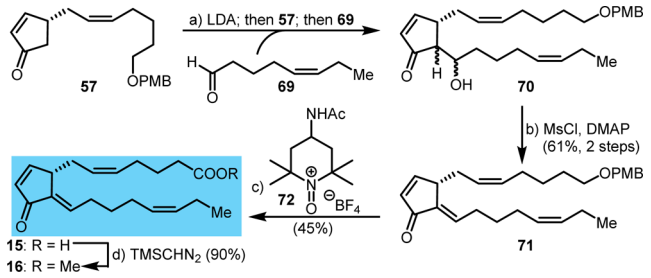
Scheme 6. Synthesis of 15-Deoxy-$\Delta^{12,14}$-PGJ$_3$ Analogue 13 and Its Methyl Ester 14[a]



[a]Reagents and conditions: (a) DDQ (1.5 equiv), 20:1 CH_2Cl_2/H_2O, 0 °C, 2 h, 94%; (b) TBSCl (1.5 equiv), imid. (3.0 equiv), CH_2Cl_2, 0 to 25 °C, 4 h, 89%; (c) LDA (2.0 equiv), THF, −78 °C; then **63** (1.0 equiv); then **64** (1.2 equiv), 15 min; (d) MsCl (2.0 equiv), DMAP (10 equiv), CH_2Cl_2, 0 to 25 °C, 6 h, 31% for two steps; (e) HF (50% aq, 50 equiv), MeCN, 0 °C, 45 min, 87%; (f) PCC (2.0 equiv), CH_2Cl_2, 25 °C, 2 h; (g) $NaClO_2$ (1.5 equiv), NaH_2PO_4 (1.5 equiv), 2-methyl-2-butene (10 equiv), *t*-BuOH, 25 °C, 30 min, 67% for two steps; (h) $TMSCHN_2$ (2 M in Et_2O, 1.5 equiv), 3:2 C_6H_6/MeOH, 25 °C, 30 min, 90%; TBSCl = *tert*-butyldimethylsilyl chloride, imid. = 1*H*-imidazole.

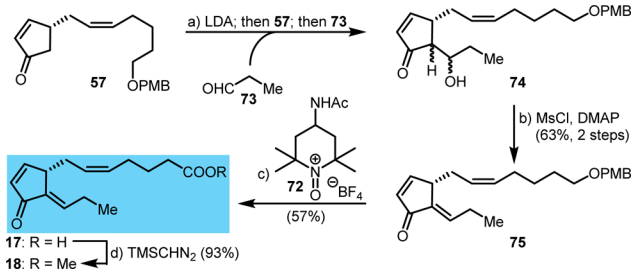
Scheme 7. Synthesis of 15-Deoxy-Δ^{12}-PGJ$_3$ (15) and Its Methyl Ester 16[a]



[a]Reagents and conditions: (a) LDA (2.0 equiv), THF, −78 °C; then **57** (1.0 equiv); then **69** (1.2 equiv), 15 min; (b) MsCl (2.0 equiv), DMAP (10 equiv), CH_2Cl_2, 0 to 25 °C, 6 h, 61% for two steps; (c) 4-(acetylamino)-2,2,6,6-tetramethyl-1-oxo-piperidinium tetrafluoroborate (6.0 equiv), 9:1 $MeCN/H_2O$, 25 °C, 30 min, 45%; (d) $TMSCHN_2$ (2 M in Et_2O, 2.0 equiv), 3:2 C_6H_6/MeOH, 25 °C, 30 min, 90%.

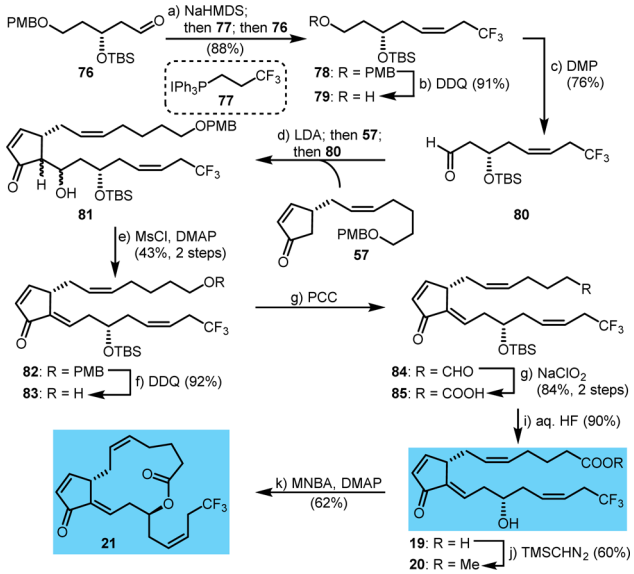
for biological comparison with their counterpart Δ^{12}-PGJ$_3$ compounds. The first task was the construction of key building block **112** (Scheme 12a). Its synthesis began with TMS−acetylene which reacted in the presence of *n*-BuLi with readily available epoxide **104**[14] to afford alcohol **105** (88%), whose desilylation (TBAF) led to terminal acetylene **106** (94% yield). Coupling of **106** with propargyl bromide **107** in the presence of CuI and K_2CO_3 furnished bis-acetylene **108**, which was selectively reduced with $NaBH_4-H_2$ in the presence of $Ni(OAc)_2{\cdot}4H_2O$[15] to give bis-olefin **109** with both olefinic bonds formed in the desired (*Z*)-geometry[16] (77% overall yield from **106**). The hydroxy group of the latter intermediate was protected as a TBS−ether (TBSCl, 91% yield), and the resulting product (**110**) was sequentially treated with DDQ (66% yield) and DMP (80% yield) to afford required aldehyde **112** through intermediate **111**. The construction of the other required

Scheme 8. Synthesis of Δ^{12}-PGJ$_3$ Analogues 17 and 18[a]



[a]Reagents and conditions: (a) LDA (2.0 equiv), THF, −78 °C; then **57** (1.0 equiv); then C_2H_5CHO (1.5 equiv), 15 min; (b) MsCl (2.0 equiv), DMAP (10 equiv), CH_2Cl_2, 0 to 25 °C, 6 h, 63% for two steps; (c) 4-(acetylamino)-2,2,6,6-tetramethyl-1-oxo-piperidinium tetrafluoroborate (6.0 equiv), 9:1 $MeCN/H_2O$, 25 °C, 30 min, 57%; (d) $TMSCHN_2$ (2 M in Et_2O, 1.5 equiv), 3:2 C_6H_6/MeOH, 25 °C, 30 min, 93%.

Scheme 9. Synthesis of Δ^{12}-PGJ$_3$ Analogue 19, Its Methyl Ester 20, and Lactone 21[a]



[a]Reagents and conditions: (a) 3,3,3-trifluoropropylphosphonium iodide (2.0 equiv), NaHMDS (1.9 equiv), 0 to 25 °C, 1 h; then **76**, −78 to 25 °C, THF, 5 h, 88%; (b) DDQ (1.5 equiv), 20:1 CH_2Cl_2/H_2O, 0 to 25 °C, 5 h, 91%; (c) DMP (2.0 equiv), CH_2Cl_2, 0 to 25 °C, 2 h, 76%; (d) LDA (2.0 equiv), THF, −78 °C; then **57** (1.0 equiv); then **80** (1.2 equiv), 15 min; (e) MsCl (2.0 equiv), DMAP (10 equiv), CH_2Cl_2, 0 to 25 °C, 6 h, 43% for two steps; (f) DDQ (1.5 equiv), 20:1 CH_2Cl_2/H_2O, 0 °C, 2 h, 92%; (g) PCC (2.0 equiv), CH_2Cl_2, 25 °C, 2 h; (h) $NaClO_2$ (3.0 equiv), NaH_2PO_4 (3.0 equiv), 2-methyl-2-butene (30 equiv), *t*-BuOH, 25 °C, 30 min, 84% for two steps; (i) HF (50% aq, 100 equiv), MeCN, 0 °C, 45 min, 90%; (j) $TMSCHN_2$ (2 M in Et_2O, 1.5 equiv), 3:2 C_6H_6/MeOH, 25 °C, 30 min, 60%; (k) MNBA (1.4 equiv), DMAP (6.0 equiv), CH_2Cl_2, 25 °C, 17 h, 62%; NaHMDS = sodium bis(trimethylsilyl)amide, DMP = Dess−Martin periodinane.

fragment, enone **118** (containing one less carbon in its side chain than the corresponding enone for Δ^{12}-PGJ$_3$), its coupling with segment **112**, and elaboration of the product to the targeted analogues **28**−**30** are shown in Scheme 12b.

Thus, cyclopentene methyl ester **113**[1] (inconsequential mixture of diastereomers) was reduced with DIBAL to afford aldehyde **114**, which was reacted with the ylide derived from phosphonium salt **115** (NaHMDS) to furnish, after desilylation

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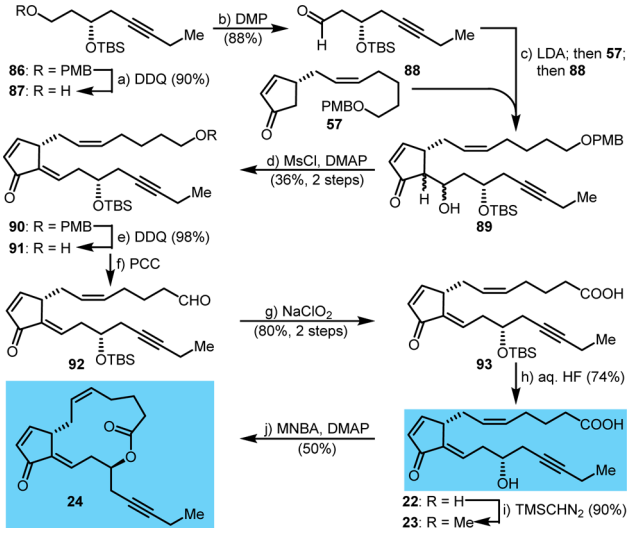
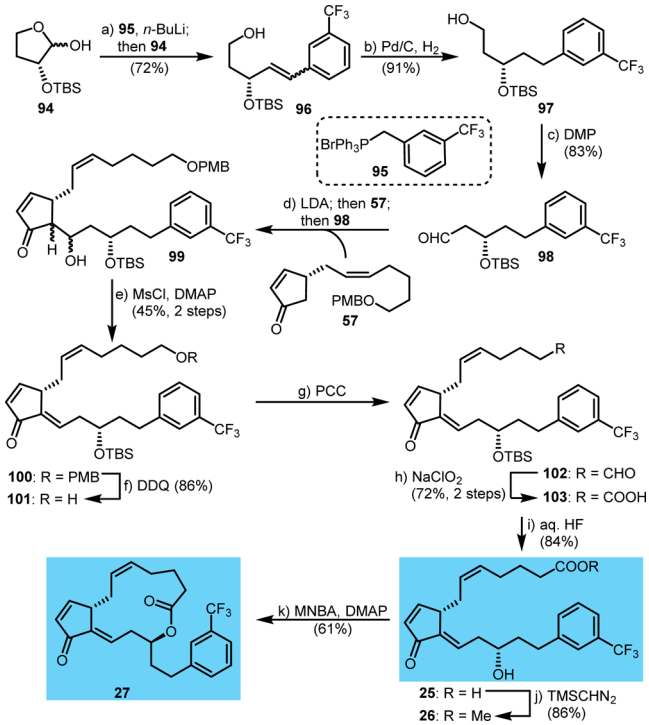
Scheme 10. Synthesis of Alkyne Δ^{12}-PGJ$_3$ Analogue 22, Its Methyl Ester 23, and Lactone 24[a]



[a]Reagents and conditions: (a) DDQ (1.5 equiv), 20:1 CH_2Cl_2/H_2O, 0 to 25 °C, 2 h, 90%; (b) DMP (1.5 equiv), CH_2Cl_2, 0 to 25 °C, 2 h, 88%; (c) LDA (2.0 equiv), THF, −78 °C; then **57** (1.0 equiv); then **88** (1.2 equiv), 15 min; (d) MsCl (2.0 equiv), DMAP (10 equiv), CH_2Cl_2, 0 to 25 °C, 6 h, 36% for two steps; (e) DDQ (2.0 equiv), 10:1 CH_2Cl_2/H_2O, 0 °C, 2 h, 98%; (f) PCC (2.0 equiv), CH_2Cl_2, 25 °C, 40 min.; (g) $NaClO_2$ (1.5 equiv), NaH_2PO_4 (1.5 equiv), 2-methyl-2-butene (10 equiv), *t*-BuOH, 25 °C, 30 min, 80% for two steps; (h) HF (50% aq, 100 equiv), MeCN, 0 °C, 30 min, 74%; (i) $TMSCHN_2$ (2 M in Et_2O, 2.0 equiv), 3:2 C_6H_6/MeOH, 25 °C, 0.5 h, 90%; (j) MNBA (1.4 equiv), DMAP (6.0 equiv), CH_2Cl_2, 25 °C, 17 h, 50%.

Scheme 11. Synthesis of Δ^{12}-PGJ$_3$ Analogue 25, Its Methyl Ester 26, and Lactone 27[a]



[a]Reagents and conditions: (a) *n*-BuLi (1.5 equiv), **95** (1.5 equiv), THF, −78 °C, 2 h; then **94** (1.0 equiv), −78 °C, 30 min, 40 °C, 24 h, 72% [(*E*)/(*Z*) = 65:35]; (b) 10% Pd/C (10 wt%), H_2, MeOH, 3 h, 91%; (c) DMP (2.0 equiv), CH_2Cl_2, 25 °C, 1 h, 83%; (d) LDA (2.0 equiv), THF, −78 °C; then **57** (1.0 equiv); then **98** (1.2 equiv), 15 min; (e) MsCl (2.0 equiv), DMAP (10 equiv), CH_2Cl_2, 0 to 25 °C, 6 h, 45% for two steps; (f) DDQ (1.5 equiv), 20:1 CH_2Cl_2/H_2O, 0 °C, 2 h, 86%; (g) PCC (2.0 equiv), CH_2Cl_2, 25 °C, 2 h; (h) $NaClO_2$ (3.0 equiv), NaH_2PO_4 (3.0 equiv), 2-methyl-2-butene (30 equiv), *t*-BuOH, 25 °C, 30 min, 72% for two steps; (i) HF (50% aq, 100 equiv), MeCN, 0 °C, 45 min, 84%; (j) $TMSCHN_2$ (2 M in Et_2O, 2.0 equiv), 3:2 C_6H_6/MeOH, 25 °C, 0.5 h, 86%; (k) MNBA (1.4 equiv), DMAP (6.0 equiv), CH_2Cl_2, 25 °C, 17 h, 61%.

(TBAF), hydroxy olefin **117** (mixture of diastereomers, 68% overall yield for the three steps from **113**). PCC oxidation of **117** led to enone **118** (92% yield) whose aldol reaction with aldehyde **112** furnished hydroxy enone **119** (mixture of diastereomers). Exposure of the latter to MsCl and Et_3N followed by treatment of the resulting mixture of mesylates (**120**) to Al_2O_3 gave the desired cross-conjugated dienone **121** in 30% overall yield for the three steps from **118**. Removal of the PMB group from **121** (DDQ, 66% yield) followed by PCC oxidation led to aldehyde **123**, whose further oxidation with $NaClO_2$ afforded TBS−ether carboxylic acid **124** in 78% overall yield from **122**. Desilylation of the latter (aq HF) afforded analogue **28**, from which methyl ester **29** was prepared by exposure to $TMSCHN_2$ (72% yield). Cyclization of **28** in the presence of MNBA and DMAP afforded lactone **30** (62% yield). Incidentally, compound **28** may found to be a naturally occurring secondary metabolite formed enzymatically from DHA by analogy to Δ^{12}-PGJ$_3$, which originates from EPA.

2.11. Synthesis of Cl-Δ^{12}-PGJ$_3$ Analogue 31, Its Methyl Ester 32, and Lactone 33. The 10-chloro series of analogues (**31**−**33**, Scheme 13) were designed expecting possible improvements in membrane permeability and potency. Their synthesis is shown in Scheme 13. Thus, PMB−ether enone **57**[1] was selectively epoxidized with H_2O_2 and KOH to afford epoxy ketone **125** whose exposure to LiCl and Amberlyst-15 furnished a mixture of PMB−ether chloroenone **126** (24% yield) and hydroxy chloroenone **127** (28% yield), the latter being easily converted to the former through the action of PMB-trichloroacetimidate and Sc(OTf)$_3$ (80% yield). Chloroenone **126** underwent aldol reaction with aldehyde **58** (LDA, −78 °C), and the resulting mixture of alcohols (**128**) so formed was treated with MsCl and DMAP to yield cross-conjugated chloroenone **129** in 45% yield for the two steps. Removal of the PMB group (DDQ, 86% yield) followed by sequential oxidation with PCC and $NaClO_2$ gave carboxylic acid **132** via aldehyde **131** in 84% yield for the two steps. Finally, the targeted compound 10-chloro-Δ^{12}-PGJ$_3$ (**31**, aq HF, 88% yield), its methyl ester (**32**, $TMSCHN_2$, 56% yield) and lactone (**33**, MNBA, DMAP, 73% yield) were generated from **132** by the standard conditions as shown in Scheme 13.

2.12. Synthesis of 8-Methyl-Δ^{12}-PGJ$_3$ Methyl Esters 34 and 36, and Lactones 35 and 37. Scheme 14 depicts the synthesis of the 8-methyl-Δ^{12}-PGJ$_3$ methyl ester (**34** and **36**) and lactone (**35** and **37**) analogues. The required 8-methyl (PG numbering) enone **137** was prepared from (+)-menthol vinylogous ester **133**[1] through a three-step sequence involving methylation (LDA, DMI, MeI, 76% yield), alkylation with allylic bromide **135** (LDA, DMI, 73% yield), and DIBAL-H-induced removal of the chiral auxiliary (76% yield) affording racemic **137** via intermediate **136** (ca. 55:45 dr). Processing enone **137** through the standard aldol-mesylation/elimination-oxidation sequence as summarized in Scheme 14 led to TBS−ether carboxylic acid **141** (mixture of diastereomers ca. 66:33 dr). The

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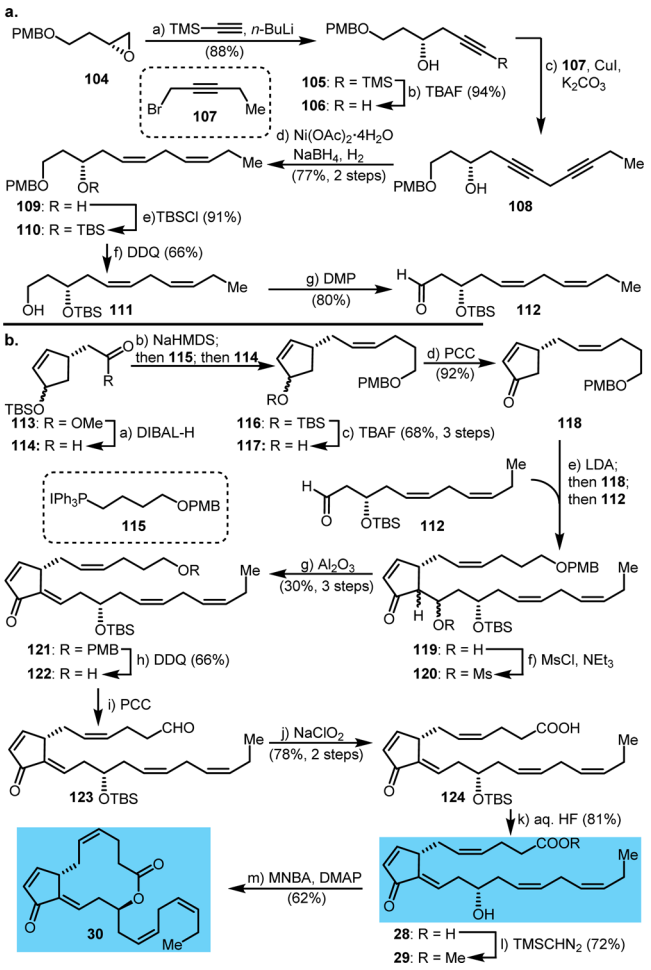
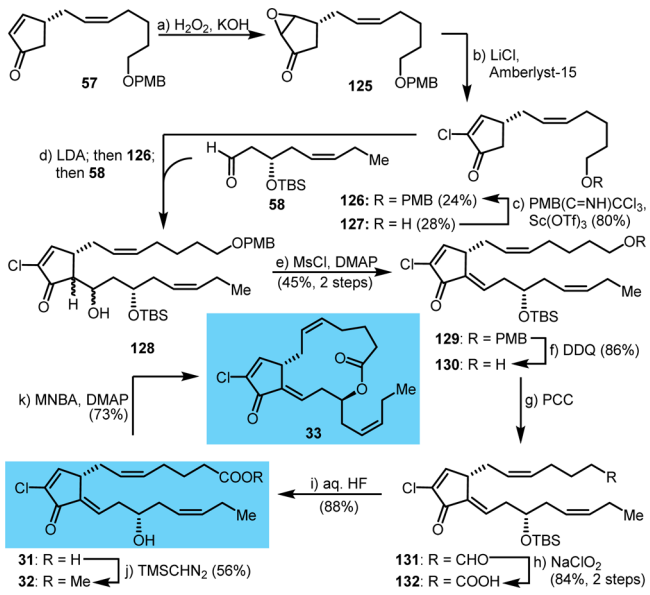
Scheme 12. Synthesis of Δ^{12}-PGJ$_3$ Analogue 28, Its Methyl Ester 29, and Lactone 30[a]



[a]Panel (a): Synthesis of aldehyde **112**: Reagents and conditions: (a) TMS—acetylene (1.5 equiv), *n*-BuLi (1.3 equiv), $BF_3{\cdot}Et_2O$ (1.3 equiv), −78 to 25 °C, 2 h, 88%; (b) TBAF (1.0 M in THF, 1.2 equiv), THF, 0 to 25 °C, 2 h, 94%; (c) **107** (1.2 equiv), K_2CO_3 (1.3 equiv), CuI (1.3 equiv), NaI (1.3 equiv), DMF, 25 °C, 15 h; (d) $Ni(OAc)_2{\cdot}4H_2O$ (0.32 equiv), $NaBH_4$ (0.77 equiv), 1,2-diaminoethane (3.6 equiv), H_2, EtOH, 25 °C, 18 h, 77% for two steps; (e) TBSCl (1.5 equiv), imid. (3.0 equiv), CH_2Cl_2, 12 h, 91%; (f) DDQ (1.5 equiv), 10:1 CH_2Cl_2/H_2O, 0 °C, 105 min, 66%; (g) DMP (1.5 equiv), CH_2Cl_2, 0 to 25 °C, 1.5 h, 80%. Panel (b): Synthesis of Δ^{12}-PGJ$_3$ analogue **28**, methyl ester **29**, and lactone **30**: Reagents and conditions: (a) DIBAL-H (1.1 equiv), CH_2Cl_2, −78 °C, 45 min; (b) **115** (1.5 equiv), NaHMDS (2.0 equiv), THF, 0 to 25 °C, 15.5 h; (c) TBAF (1.2 equiv), THF, 0 to 25 °C, 3 h, 68% for three steps; (d) PCC (1.9 equiv), CH_2Cl_2, 25 °C, 2 h, 92%; (e) LDA (2.2 equiv), then **112** (1.3 equiv), THF, −78 °C, 30 min; (f) MsCl (5.0 equiv), Et_3N (10 equiv), CH_2Cl_2, 0 °C, 30 min; (g) Al_2O_3 (21 equiv), CH_2Cl_2, 25 °C, 6 h, 30% for three steps; (h) DDQ (1.7 equiv), 10:1 CH_2Cl_2/H_2O, 0 °C, 105 min, 66%; (i) PCC (2.0 equiv), CH_2Cl_2, 25 °C, 75 min; (j) 2-methyl-2-butene (10 equiv), NaH_2PO_4 (1.5 equiv), $NaClO_2$ (1.5 equiv), *t*-BuOH, 25 °C, 30 min, 78% for two steps; (k) aq HF (ca. 100 equiv), MeCN, 0 °C, 75 min, 81%; (l) $TMSCHN_2$ (2 M in Et_2O, 2.0 equiv), 3:2 C_6H_6/MeOH, 25 °C, 30 min, 72%; (m) MNBA (1.4 equiv), DMAP (6.0 equiv), CH_2Cl_2, 25 °C, 17 h, 62%; TBAF = tetra-*n*-butylammonium fluoride.

sluggishness and low yield of the aldol reaction in this case is attributed to steric hindrance introduced by the C8 quaternary center adjacent to the reactive site. Desilylation of **141** led to hydroxy acid **142** as a mixture of diastereomers (75% yield, ca.

Scheme 13. Synthesis of Cl-Δ^{12}-PGJ$_3$ Analogue 31, Its Methyl Ester 32, and Lactone 33[a]



[a]Reagents and conditions: (a) H_2O_2 (2.0 equiv), KOH (10% in H_2O, 0.2 equiv), MeOH, −20 °C, 6 h; (b) LiCl (10.0 equiv), Amberlyst-15 (200 wt%), MeCN, 25 °C, 24 h, **126** (24%) and **127** (28%); (c) PMBOC(=NH)CCl$_3$ (2.0 equiv), $Sc(OTf)_3$ (0.1 equiv), CH_2Cl_2, 25 °C, 4 h, 80%; (d) LDA (2.0 equiv), THF, −78 °C; then **126** (1.0 equiv); then **58** (1.3 equiv), 15 min; (e) MsCl (2.0 equiv), DMAP (10 equiv), CH_2Cl_2, 0 to 25 °C, 12 h, 45% for two steps; (f) DDQ (2.0 equiv), 20:1 CH_2Cl_2/H_2O, 0 °C, 2 h, 86%; (g) PCC (2.0 equiv), CH_2Cl_2, 25 °C, 2 h; (h) $NaClO_2$ (3.0 equiv), NaH_2PO_4 (3.0 equiv), 2-methyl-2-butene (30 equiv), *t*-BuOH, 25 °C, 30 min, 84% for two steps; (i) HF (50% aq, 100 equiv), MeCN, 0 °C, 45 min, 88%; (j) $TMSCHN_2$ (2 M in Et_2O, 2.0 equiv), 3:2 C_6H_6/MeOH, 25 °C, 30 min, 56%; (k) MNBA (1.5 equiv), DMAP (4.0 equiv), CH_2Cl_2, 25 °C, 12 h, 73%.

65:35 dr) whose chromatographic separation proved challenging. The difficulties of separating the C8-epimers of carboxylic acid **142** were reduced upon methylation ($TMSCHN_2$, 82% yield) to produce methyl ester analogues **34** (57%) and **36** (25%) (chromatographically separated on silica gel) or upon lactone formation (MNBA, DMAP, 59% yield) to afford lactones **35** (38%) and **37** (21%) (chromatographically separated on silica gel). The absolute configuration of the C8-stereocenter in this series of compounds was not discernible from the NMR spectral data and, therefore, the depicted structures should be considered interchangeable (i.e., **34** or **36**; **35** or **37**).

2.13. Synthesis of 15-Fluoro-Δ^{12}-PGJ$_3$ (38) and Its Methyl Ester 39. The 15-fluoro-Δ^{12}-PGJ$_3$ analogues **38** and **39** were prepared from enone **57**[1] and aldehyde *ent*-**58**,[5] the latter chosen so as to allow for the anticipated inversion of configuration at the fluorination stage as shown in Scheme 15. Building blocks **57**[1] and *ent*-**58**[5] were united through the standard aldol protocol to afford diastereomeric mixture **143**, which was converted to cross-conjugated enone **144** through sequential treatment with MsCl, Et_3N and Al_2O_3. The latter was desilylated ($3HF{\cdot}Et_3N$) to give alcohol **145** whose exposure to PhenoFluor at 80 °C furnished fluoride **146** (35% yield) with (presumed) inversion of configuration at C15.[17] Precursor **146** was converted in one step to the targeted 15-fluoro-Δ^{12}-PGJ$_3$ analogue **38** with piperidinium tetrafluoroborate **72**[8] in 45% yield. 15-Fluoro-Δ^{12}-PGJ$_3$ methyl ester (**39**) was prepared from

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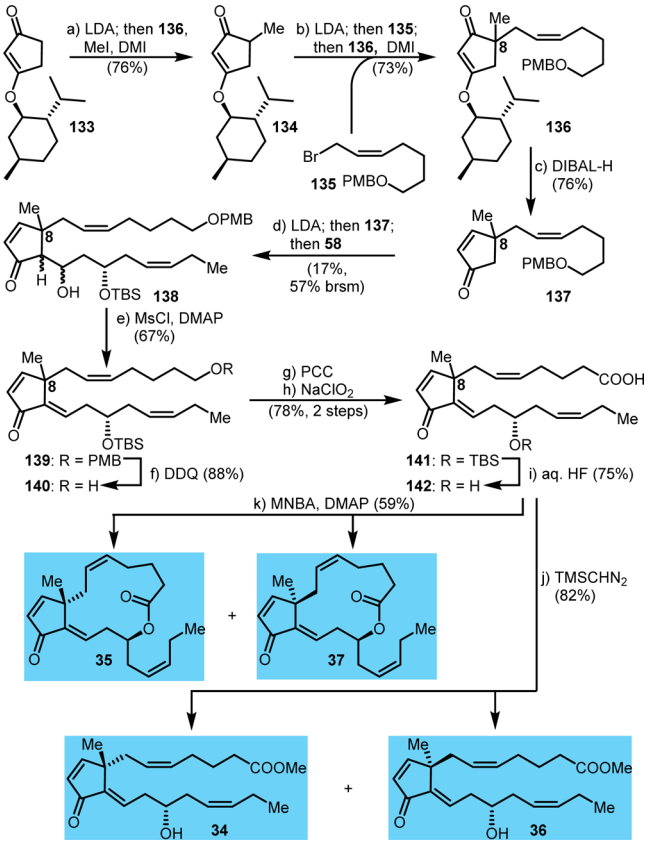
Scheme 14. Synthesis of 8-Methyl-Δ^{12}-PGJ$_3$ Methyl Esters 34 and 36, and Lactones 35 and 37[a]



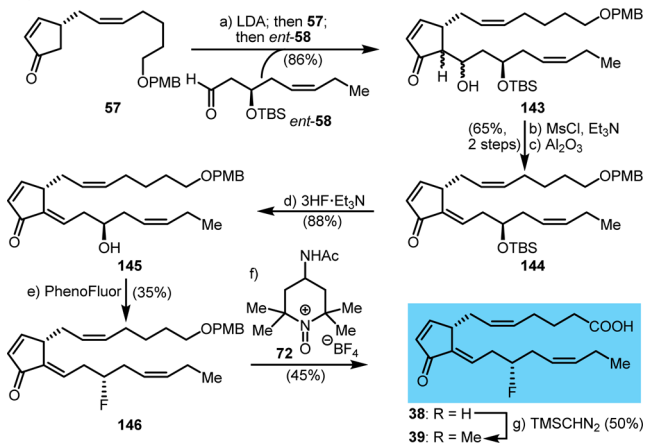
[a]Reagents and conditions: (a) LDA (1.05 equiv), THF, −78 °C; then **133** (1.0 equiv), 0.5 h, then MeI (1.3 equiv), DMI, 12 h, 76%; (b) LDA (1.05 equiv), THF, −78 °C; then **134** (1.0 equiv); then **135** (1.3 equiv), DMI, 12 h, 73% (mixture of diastereomers, ca. 55:45 dr); (c) DIBAL-H (1.5 equiv), CH_2Cl_2, −10 °C, 6 h, 76%; (d) LDA (2.0 equiv), THF, −78 °C; then **137** (1.0 equiv); then **58** (1.2 equiv), 30 min, 17% (57% brsm); (e) MsCl (2.0 equiv), DMAP (10 equiv), CH_2Cl_2, 0 to 25 °C, 6 h, 67% for two steps (mixture of diastereomers, ca. 67:33 dr); (f) DDQ (2.0 equiv), 20:1 CH_2Cl_2/H_2O, 0 °C, 2 h, 88% (mixture of diastereomers, ca. 68:32 dr); (g) PCC (2.0 equiv), CH_2Cl_2, 25 °C, 2 h; (h) $NaClO_2$ (3.0 equiv), NaH_2PO_4 (3.0 equiv), 2-methyl-2-butene (10 equiv), *t*-BuOH, 25 °C, 30 min, 78% for two steps (mixture of diastereomers, ca. 66:33 dr); (i) HF (50% aq, 50 equiv), MeCN, 0 °C, 45 min, 75% (mixture of diastereomers, ca. 65:35 dr); (j) $TMSCHN_2$ (2 M in Et_2O, 1.5 equiv), 3:2 C_6H_6/MeOH, 25 °C, 0.5 h, 82% (57% for **34**; 25% for **36**); (k) MNBA (1.4 equiv), DMAP (6.0 equiv), CH_2Cl_2, 25 °C, 12 h, 59% (38% for **35**; 21% for **37**); DMI = 1,3-dimethyl-2-imidazolidinone.

38 by treatment with $TMSCHN_2$ (50% yield) as summarized in Scheme 15.

Truncated compounds **40**−**43** (Figure 1) were also synthesized (see Supporting Information) in order to test the effects of shorter "top" side chains on the biological activity in the series, while 17,18-dihydro lactone analogue **44** was constructed (see Supporting Information) to probe the effect of another "lower" side chain modification.

2.14. Biological Evaluation of the Δ^{12}-PGJ$_3$ Analogues. The synthesized Δ^{12}-PGJ$_3$ (**1**) and Δ^{12}-PGJ$_3$ analogues (**2**−**44**, *ent*-**1**, *ent*-**2**, and *ent*-**11**) were evaluated for their cytotoxicities against the NIH-60 Screen panel of human cancer cell lines, including leukemia, non-small cell lung, colon, central nervous system, melanoma, ovarian, renal, breast, and prostate cancer cell

Scheme 15. Synthesis of 15-Fluoro-Δ^{12}-PGJ$_3$ (38) and Its Methyl Ester 39[a]



[a]Reagents and conditions: (a) LDA (2.0 equiv), THF, −78 °C; then **57** (1.0 equiv), 30 min; then *ent*-**58** (1.2 equiv), 30 min, 86%; (b) MsCl (5.0 equiv), Et_3N (10 equiv), CH_2Cl_2, 0 °C, 5 min; (c) Al_2O_3 (42 equiv), CH_2Cl_2, 25 °C, 8 h, 65% for two steps; (d) 3HF·Et_3N (23 equiv), THF, 0 to 25 °C, 3 d, 88%; (e) PhenoFluor (6.0 equiv), KF (9.0 equiv), *i*-Pr_2NEt (9.0 equiv), toluene, 80 °C, 2.5 h, 35%; (f) 4-(acetylamino)-2,2,6,6-tetramethyl-1-oxo-piperidinium tetrafluoroborate (6.0 equiv), 9:1 MeCN/H_2O, 25 °C, 35 min, 45%; (g) $TMSCHN_2$ (2 M in Et_2O, 1.5 equiv), 3:2 C_6H_6/MeOH, 25 °C, 35 min, 50%; PhenoFluor = 1,3-bis(2,6-diisopropylphenyl)-2,2-difluoro-2,3-dihydro-1*H*-imidazole.

lines. Selected data are shown in Table 1 (for full data, see Supporting Information). Compounds **1**, **5**, *ent*-**1**, **13**, **17**, **19**, **22**, **31**, **34**−**36**, and **40**−**43** did not advance beyond the one-dose assay (10 μM) as they did not show significant cytotoxicities (see Table 1), whereas the remaining compounds, having exhibited promising potencies, were tested at lower concentrations. As shown in Table 1, among the most potent of these analogues against the tested cell lines were the following: dimethyl amide **9** (melanoma, GI_{50} = 0.356 μM); methyl esters **29** (melanoma, GI_{50} = 0.210 μM), and **32** (leukemia, GI_{50} = 0.0893 μM); lactones **11** (colon cancer, GI_{50} = 0.201 μM), *ent*-**11** (colon cancer, GI_{50} = 0.133 μM), **24** (breast cancer, GI_{50} = 0.203 μM), **33** (leukemia, GI_{50} = 0.0753 μM), and **44** (melanoma, GI_{50} = 0.084 μM). Interestingly, Δ^{12}-PGJ$_3$ macrolactone **11** demonstrated no toxicity in athymic nude mice up to 200 mg/kg per dose (intraperitoneally administered), a promising result that allowed it to advance to *in vivo* efficacy studies in mice (NCI, see Supporting Information).

A selected number of compounds were also assayed for their cytotoxicities against cancer cell lines HEK 293T (human embryonic kidney cell line), MES SA (uterine sarcoma cell line), and MES SA DX (MES SA cell line with marked multidrug resistance). The results are summarized in Table 2. The most potent proved to be 10-chloro-Δ^{12}-PGJ$_3$ derivatives **32** (IC_{50} = 0.26 μM against HEK 293T) and **33** (IC_{50} = 0.32 μM against MES SA DX); lactones **11** (IC_{50} = 0.074 μM against MES SA DX) and **24** (IC_{50} = 0.31 μM against MES SA DX); amides **7** (IC_{50} = 0.56 μM against HEK 293T) and **9** (IC_{50} = 0.32 μM against HEK 293T), and methyl ester **2** (IC_{50} = 0.57 μM against MES SA).

2.15. Nuclear Export Studies. Compounds **2**, **11**, **21**, and **27** were tested for their ability to inhibit nuclear export, motivated by reported activities of the structurally related 15-

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Table 1. Selected NCI-60 Cytotoxicity Screen Human Cancer Cell Line Panel Data (GI_{50} in μM)[a] for Δ^{12}-PGJ_3 (1) and Analogues 2−44, *ent*-1, *ent*-2, and *ent*-11[b]

compound	one dose[c]	leukemia	non-small cell lung cancer	colon cancer	CNS cancer	melanoma	ovarian cancer	renal cancer	breast cancer	prostate cancer
1	58.29	-	-	-	-	-	-	-	-	-
2	-	0.368	1.03	0.538	1.63	0.237	0.692	1.75	0.376	2.70
3	-	0.371	1.25	0.572	0.480	0.180	0.483	1.76	0.372	1.58
4	-	0.534	0.753	0.590	0.581	0.536	0.671	0.827	0.750	0.836
5	98.69	-	-	-	-	-	-	-	-	-
6	-	1.14	1.30	1.02	1.50	0.350	1.26	1.62	0.626	2.15
7	-	0.957	1.54	0.612	1.30	0.317	1.20	1.52	0.455	1.95
8	-	2.09	1.59	1.73	2.61	1.06	2.63	1.94	1.67	2.52
9	-	0.376	1.33	0.440	1.32	0.356	0.471	0.504	0.368	1.78
10	-	2.44	1.76	1.99	3.35	1.44	3.19	2.37	2.28	3.83
11	-	0.309	0.415	0.201	0.773	0.219	0.343	0.206	0.387	1.42
12	-	1.97	1.74	1.58	2.03	1.34	3.22	1.63	0.544	3.32
ent-**1**	90.92	-	-	-	-	-	-	-	-	-
ent-**2**	-	0.461	1.65	0.498	3.18	0.329	3.10	1.16	2.12	3.20
ent-**11**	-	0.170	0.231	0.133	0.365	0.145	0.342	0.183	0.356	0.362
13	98.44	-	-	-	-	-	-	-	-	-
14	-	0.398	1.78	0.631	2.35	0.417	1.54	1.64	0.500	2.44
15	-	1.92	2.02	1.80	3.52	1.46	2.96	1.89	3.07	3.64
16	-	2.43	1.91	2.10	3.10	1.59	3.30	2.04	2.69	3.14
17	78.37	-	-	-	-	-	-	-	-	-
18	-	0.519	1.56	0.447	2.60	0.505	2.88	2.03	1.21	3.02
19	93.39	-	-	-	-	-	-	-	-	-
20	-	0.345	1.06	1.07	1.79	0.243	1.31	1.70	0.393	2.79
21	-	0.267	0.257	0.299	0.401	0.280	0.423	0.298	0.292	0.574
22	100.62	-	-	-	-	-	-	-	-	-
23	-	0.278	0.673	0.235	0.695	0.754	1.16	0.864	1.43	0.297
24	-	0.275	0.663	0.430	0.706	0.559	0.568	0.730	0.203	1.35
25	-	0.465	1.35	1.27	1.55	1.29	1.99	1.65	0.444	2.43
26	-	0.975	1.95	1.67	2.06	0.955	1.97	1.73	0.775	2.59
27	-	0.397	1.23	0.326	1.26	0.439	0.495	1.19	0.287	1.66
28	-	2.17	1.71	2.08	3.54	1.74	3.23	2.47	2.32	5.17
29	-	0.281	1.03	0.494	1.45	0.210	0.774	1.55	0.329	2.53
30	-	0.847	0.524	0.634	0.995	0.563	0.747	0.686	1.18	0.837
31	49.47	-	-	-	-	-	-	-	-	-
32	-	0.0893	0.271	0.275	0.339	0.258	0.347	0.378	0.185	0.539
33	-	0.0753	0.156	0.0995	0.214	0.159	0.143	0.300	0.0836	0.346
34	100.52	-	-	-	-	-	-	-	-	-
35	93.16	-	-	-	-	-	-	-	-	-
36	101.45	-	-	-	-	-	-	-	-	-
37[d]	-	-	-	-	-	-	-	-	-	-
38	-	1.04	4.58	1.34	4.01	3.77	1.21	4.99	1.32	6.09
39	-	1.06	1.32	0.880	1.86	0.854	1.71	1.25	1.02	1.74
40	100.98	-	-	-	-	-	-	-	-	-
41	100.84	-	-	-	-	-	-	-	-	-
42	96.77	-	-	-	-	-	-	-	-	-
43	94.50	-	-	-	-	-	-	-	-	-
44	-	0.106	0.090	0.091	0.116	0.084	0.149	0.090	0.165	0.088

[a]GI_{50} = Concentration of compound required to inhibit growth by 50%. [b]See Supporting Information for complete NCI-60 screen data. [c]Mean growth % at 10 μM. [d]Compound **37** was submitted to NCI after testing of compounds **34**, **35**, and **36** and was not chosen to be tested.

deoxy-$\Delta^{12,14}$-PGJ_2.[18] All compounds inhibited the nuclear export of a translocation biosensor at a concentration of 50 μM (Figure S1). This effect was confirmed by monitoring the intracellular localization of the full length protein TFIIA fused to GFP (Figure 2A). The compounds exhibited comparable potencies to those reported for 15-deoxy-$\Delta^{12,14}$-PGJ_2 in both assays.[18] The inhibitory effect was completely revoked upon addition of dithiothreitol (Figure S2), indicating a mechanism involving a nucleophilic attack by a thiol (e.g., cysteine residue) on the cross-conjugated enone moiety, most likely at the endocyclic C9 position.[19] The export of both substrates, the translocation biosensor and TFIIA, is mediated by the receptor Crm1. Cys528 of Crm1, located in the binding pocket for export substrates, is the site of attack of covalently binding nuclear export inhibitors like leptomycin B and ratjadones.[20] To investigate whether **2**, **11**, **21**, and **27** may also act through binding to Cys528, two peptides were synthesized. The first one comprised residues 523−531 of Crm1 (DLLGL**C**EQK), while the second contained a cysteine to serine exchange at position 528 (DLLGL**S**EQK). For all four compounds, covalent binding to DLLGL**C**EQK was detected by

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Table 2. Cytotoxicity Data Against Cancer Cell Lines HEK 293T, MES SA, and MES SA DX[a] for Δ^{12}-PGJ$_3$ and Selected Analogues (IC_{50} Values in μM)[b]

compound	HEK 293T	MES SA	MES SA DX
1	>5	>5	>5
2	0.583	0.569	0.615
3	~0.955	1.288	1.014
7	0.559	0.571	0.806
8	2.080	2.608	4.045
9	0.323	0.484	0.531
10	2.902	22.150	67.950
11	0.080	0.113	0.074
ent-**11**	0.490	0.435	0.346
15	>5	>5	>5
16	1.243	1.654	1.760
17	>5	>5	>5
18	0.734	0.681	0.698
21	0.660	1.458	0.861
24	0.418	0.468	0.314
27	~0.945	1.450	1.105
30	0.778	1.438	1.430
32	0.262	0.428	0.381
33	0.370	0.410	0.316
35	>10	>10	>10
37	81.500	>10	>10
40	>25	>25	>25

[a]IC_{50} = 50% inhibitory concentration of compound against cell growth; MES SA = uterine sarcoma cell line; MES SA DX = MES SA cell line with marked multidrug resistance; HEK 293T = human embryonic kidney cell line. [b]See Supporting Information for further details. These studies were carried out at Stemcentrx.

mass spectrometric analysis (Figures 2B, S3–S6, and Table ST1). In contrast, no binding to DLLGL**S**EQK was observed. Binding to Cys528 of Crm1 was validated in a cellular environment by monitoring the decreased co-localization between Crm1 and HIV-1Rev, but not between mutant $Crm1_{C528S}$ and HIV-1Rev, upon compound treatment (Figure 2C). Similarly, expression of $Crm1_{C528S}$-HA but not Crm1-HA rescued nuclear export of the translocation biosensor upon treatment with compound **2** (Figure S8). Thus, it was shown that **2**, **11**, **21**, and **27** acted as inhibitors of nuclear export through covalent bond formation at Cys528 of the export receptor Crm1.

The IC_{50} values representing the cytotoxic effects of the tested compounds differ significantly from the concentrations needed for inhibiting nuclear export. Because nuclear export inhibition was determined after 1.5 h of incubation, whereas cytotoxicity was assessed after 48 h of treatment, the higher cytotoxic potency may arise from the extended time for the compound to exert an effect. Nevertheless, nuclear export inhibition must account at least in part for the compound's cytotoxic effects, as this consistency has been previously reported for various nuclear export inhibitors (reviewed in ref. 21). It is also of note that Δ^{12}-PGJ$_3$ was reported to activate the ATM/p53 pathway of apoptosis in leukemia stem cells.[3a] This effect may also be expected for the structurally related compounds described herein, providing an additional mode of action for their cytotoxic effects.

2.16. Structure–Activity Relationships (SARs). From the gathered biological data, a number of useful path-pointing structure–activity relationships (SARs) were formed. Thus, esterification at the C1 position of Δ^{12}-PGJ$_3$ led to increase of potency as indicated by the GI_{50} (Table 1) and IC_{50} (Table 2) values for esters **2**–**4** and **10** (compared to those for **1**), with the shorter O-chain (i.e., OMe, O*i*Pr, compounds **2** and **3**) being optimal. These observations suggest cell permeability increase imparted by the ester groups in compounds **2** and **3** as opposed to the carboxylic acid moiety, although the length and steric demand of the side-chain seems to also play a moderating role as shown by the reversal of the trend with analogues **4**, **5**, and **10**. Amide groups at C1 also seem to be tolerated and, in some cases, are even more potent than the corresponding methyl esters as demonstrated by dimethyl amide analogue **9** (see Tables 1 and 2). The most significant potency enhancement, however, was achieved through macrolactonization (C1 carboxylic acid with C15 hydroxy moieties) as demonstrated within this series of compounds (i.e., **11**, *ent*-**11**, **24**, **27**, **30**, **33**, **37**, and **44**). These results also demonstrate enhancement of potency in going from the carboxylic acid to methyl ester to the macrolactone analogues (e.g., *ent*-**1** → *ent*-**2** → *ent*-**11**; **19** → **20** → **21**). Interestingly, reduction of the carboxylic acid (**1**) to its primary alcohol counterpart (i.e., **12**) resulted in higher potencies than the parent compound possibly due to increase of lipophilicity. It is plausible that all these C1-carboxylate derivatives act as prodrugs, generating the carboxylic acid once inside the cell, although whether this group is essential for cytotoxicity is not known at this time.

The results with the enantiomeric Δ^{12}-PGJ$_3$ analogues *ent*-**1** (acid), *ent*-**2** (methyl ester), and *ent*-**11** (lactone) are of interest in that they demonstrate comparable cytotoxicity properties to those of the natural absolute configurations (i.e., **1**, **2**, and **11**), except for the cases of *ent*-**2** [showing lower potencies against breast and ovarian cancer cells than its parent counterpart (**2**), see Table 1] and *ent*-**11** [which exhibited higher potencies than its parent compound (i.e., **11**) in the majority of the assays performed (see Table 1)].

Of interest was also the fact that the C14–C15 dehydro-derivative of Δ^{12}-PGJ$_3$ methyl ester analogue **14** exhibited comparable cytotoxicities to those of the parent compound (i.e., **2**, see Table 1) but higher potencies than Δ^{12}-PGJ$_3$-C15-deoxy analogue **16**. More interesting was the observation that truncation of the lower side chain by removing its C16–C20 segment and the C15-hydroxy group as in Δ^{12}-PGJ$_3$ methyl ester analogue **18** resulted only in minor loss of activity compared to that of Δ^{12}-PGJ$_3$ methyl ester (**2**). Even more gratifying was the realization that replacement of the terminal methyl group of the lower side chain with a trifluoromethyl group (i.e., compounds **19**–**21**) led to an increase of potency in most of the tested cell lines compared to those of the parent compounds (i.e., **1**, **2**, and **11**, see Table 1). Furthermore, substitution of the C17–C18 olefinic bond with an acetylenic linkage (i.e., compounds **22**–**24**), installment of a *m*-trifluoromethyl aryl moiety (i.e., compounds **25**–**27**), or replacement of the C15-hydroxy group with a fluoride residue (i.e., compounds **38**, **39**) within the Δ^{12}-PGJ$_3$ molecule led to similar or slightly higher potencies. These data lead to the conclusion that a wide variety of modifications of the lower side chain can be tolerated without significant loss of activity. In fact, in some cases such changes may lead to moderate increase in potency as demonstrated with lactone **21** or even more significant enhancement of potency (see below). In contrast to the lower side chain modifications, changes in the top side chain such as truncation with attachment point modification (to the cyclopentenone moiety, e.g., compounds **40**–**43**) led to complete loss of activity as seen in Table 1. A similar loss of potency was seen by introduction of a

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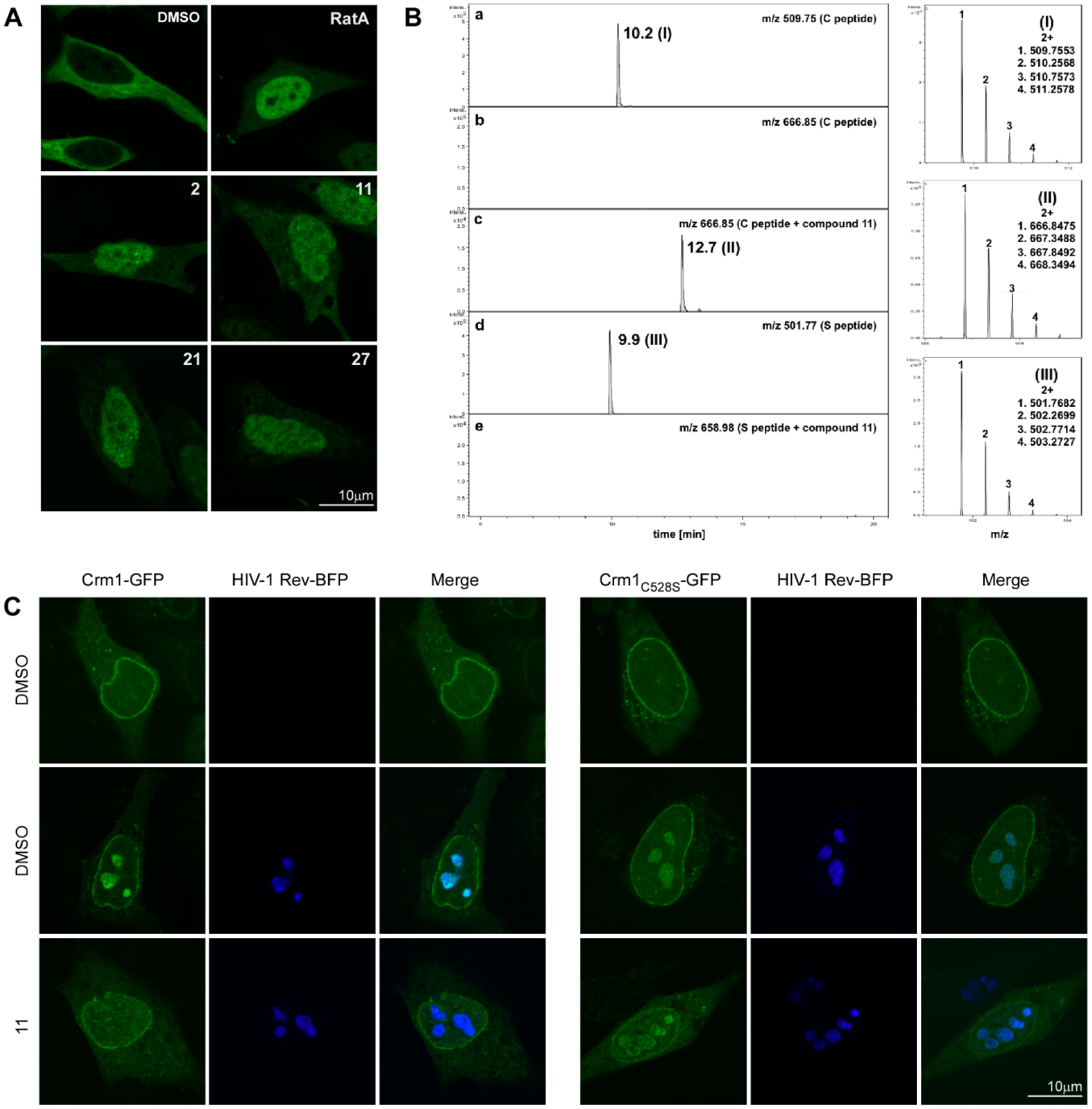

Figure 2. Compounds **2**, **11**, **21**, and **27** inhibit Crm1-dependent nuclear export; HeLa cells transiently expressing TFIIA-GFP were treated with compounds **2**, **11**, **21**, and **27** (50 μM) for 1.5 h. The intracellular localization of TFIIA was evaluated by fluorescence microscopy after fixation (A). The binding of **11** to a peptide comprising amino acids 523−531 of Crm1 (DLLGLCEQK; C-peptide) and a control peptide (DLLGLSEQK, S-peptide) was evaluated by liquid chromatography−mass spectrometry. Depicted are extracted ion chromatograms for the doubly protonated ions of the peptide (509.75 Da for C-peptide and 501.77 Da for S-peptide) and peptide−compound conjugate masses (666.85 Da for C-peptide−**11** and 658.98 Da for S-peptide−**11**) and the corresponding high resolution mass spectra (B); co-localization of Crm1 and HIV-1 Rev is disabled by the compounds (C). HeLa cells were co-transfected with plasmids encoding for Crm1-GFP and HIV-1 Rev-BFP or $Crm1_{C528S}$-GFP and HIV-1 Rev-BFP.[22] The day after transfection compounds **2**, **11**, **21**, and **27** were applied at a concentration of 50 μM for 1.5 h. Cells were fixed and the localization of fluorescent signals was analyzed by confocal microscopy.

methyl residue at the C8 position of Δ^{12}-PGJ_3 (i.e., compounds **34**−**37**, see Tables 1 and 2).

A most notable enhancement of activity in this series of compounds (see Figure 1) was observed upon introduction of a chlorine residue at the C10 position of the Δ^{12}-PGJ_3 molecule (analogues **31**−**33**). Indeed, 10-chloro lactone **33** exhibited the second highest potencies of the series in the NCI-60-Screen proving itself more potent than **11**. The most potent compound within this series of analogues turned out to be lactone **44**, which lacks the $\Delta^{17,18}$-olefinic double bond in the lower side chain.

Figure 3 provides a summary of the structure−activity relationships (SARs) as derived from these investigations.

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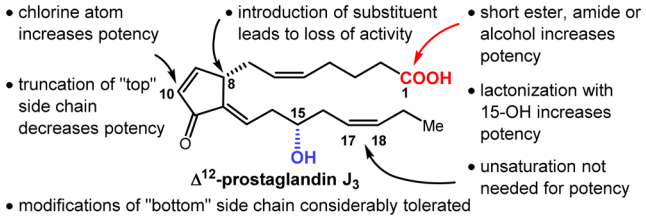

Figure 3. Structure–activity relationships (SARs) of Δ^{12}-PGJ$_3$ family of compounds.

3. CONCLUSION

Inspired by a recent report on the isolation and biological properties of Δ^{12}-prostaglandin J$_3$ (Δ^{12}-PGJ$_3$, **1**)[3] and enabled by our synthetic approaches to this naturally occurring molecule,[1,2,5] the described chemistry resulted in the chemical synthesis of a series of designed analogues (**2**–**44**, *ent*-**1**, *ent*-**2**, and *ent*-**11**). Biological evaluation of these compounds led to the identification of a number of cytotoxic agents against a variety of cancer cell lines and established valuable structure activity relationships (SARs). These investigations led to the identification of macrolactone **11** as a preclinical development drug candidate by virtue of its promising chemical and pharmacological profiles. Due to their higher potency, compounds **33** and **44** (synthesized after compound **11** was selected for early development) are also under consideration for further preclinical development. Improvement of the biomedical properties of these compounds are expected through further molecular fine-tuning based on the established SARs in this study.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/jacs.6b02075.

Experimental procedures and characterization data for all compounds; NCI-60 cytotoxicity screen and HEK 293T, MES SA, and MES A DX data (PDF)

■ AUTHOR INFORMATION

Corresponding Author

*kcn@rice.edu

Author Contributions

§K.K.P., S.R., and P.H. contributed equally to this work.

Notes

The authors declare no competing financial interest.

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