Tetrahedron Letters 55 (2014) 3713–3716

Contents lists available at ScienceDirect

Tetrahedron Letters

journal homepage: www.elsevier.com/locate/tetlet

New carbocyclic nucleosides: synthesis of carbocyclic pseudoisocytidine and its analogs

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ARTICLE INFO

Article history:
Received 6 February 2014
Revised 18 April 2014
Accepted 7 May 2014
Available online 20 May 2014

Keywords:
Nucleoside analogs
Pseudoisocytidine
Diastereoselective synthesis
Glycosylases
NEIL1

ABSTRACT

Cyclopentane-containing nucleoside analogs with a C—C connection between the (heterocyclic) base and the carbocyclic scaffold are quite rare. Herein, we report the synthesis of previously unknown racemic carbocyclic pseudoisocytidine and its analogs, which were prepared in 13 steps from commercially available materials. Pseudoisocytidine and its sulfur analog were moderately active against the mantle cell lymphoma cell line, JVM-3. We also prepared a versatile cyclopentanone intermediate, which can be converted into novel carbocyclic nucleosides via highly stereoselective addition of organometallic nucleophiles; the adduct with phenyllithium, the stereochemistry of which was unambiguously confirmed by X-ray crystallography, inhibits glycosylase NEIL1 in a dose-dependent manner.

Nucleoside analogs represent a diverse group of organic compounds. Appropriate modifications of the nucleoside scaffold can result in significantly altered biological activity.[1] As natural nucleosides contain the relatively labile aminal motif (structure **A** in Fig. 1), significant effort has been invested in order to identify more stable analogs.

One strategy consists of replacing the tetrahydrofuran ring with an appropriate carbocyclic isostere. The resulting carbanucleosides have been synthesized by diverse synthetic methods,[2] and in many cases, it has been observed that the tetrahydrofuran ring can be replaced with cyclopentane (structure **B** in Fig. 1) without a significant loss of biological activity.[3] Naturally occurring representatives of this series include aristeromycin and its unsaturated analog (−)-neplanocin A.[4] Another strategy is based on attachment of the heterocyclic base to the tetrahydrofuran via a C—C bond linkage (structure **C** in Fig. 1). While the resulting C-nucleosides are in general more stable than natural nucleosides, the synthesis of even relatively simple systems (e.g., tiazofurin and its analogs) in this series is often not trivial.[5] In addition, some C-nucleosides can still undergo ring-opening of the furan (see below). Structure **D** in Figure 1 combines elements of structures **B** and **C**: carbocyclic C-nucleosides with a C—C connection between the (heterocyclic) base and the carbocyclic scaffold. It is conceivable that, at least in some cases, these compounds might be more robust versions of nucleoside analogs **B** and **C**. Furthermore, the installation of certain substituents (e.g., R = OH) is meaningful only in this series, as this would lead to chemically unstable ketals and aminals in the other series. Interestingly, compounds with general structure **D** (where R = H) are quite rare[6] and we have been unable to find any analogs of type **D** (containing R = OH) with nucleoside-like substitution patterns.

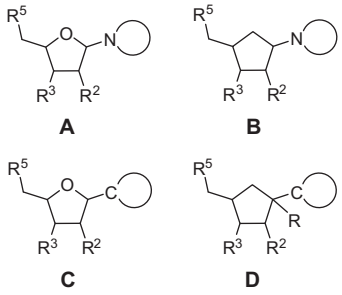

Figure 1. The common scaffold of natural nucleosides (**A**) and the structures of their analogs **B**, **C**, and **D**.

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http://dx.doi.org/10.1016/j.tetlet.2014.05.030
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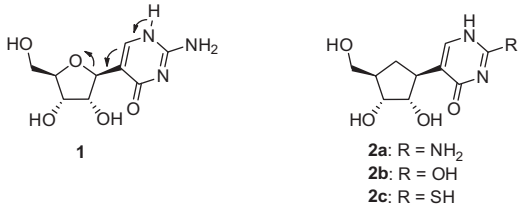


Scheme 1. Opening of pseudoisocytidine **1** and the structures of its direct carbocyclic analog **2a** and related compounds **2b** and **2c**.

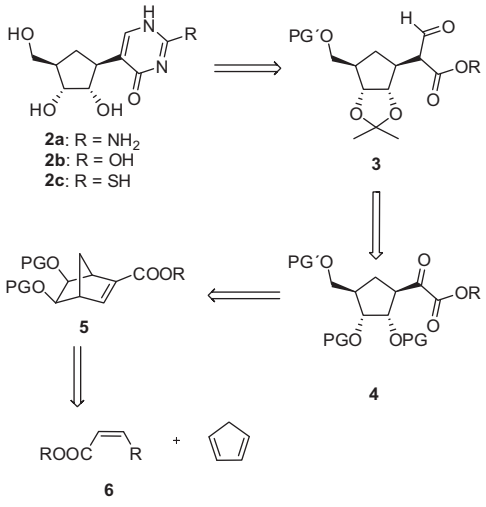
One attractive biologically active candidate for the tetrahydrofuran-cyclopentane replacement is pseudoisocytidine (**1**), which has been shown to be active against cytarabine-resistant leukemias,[7] but hepatotoxic in vivo,[8] which may be the result of opening of the tetrahydrofuran core (Scheme 1).[9]

The direct carbocyclic analog **2a** cannot undergo such a ring-opening process and its toxicological profile might therefore be superior to that of pseudoisocytidine, while its biological activity could be retained (Scheme 1). Herein, we report the first synthesis of the previously unknown carbocyclic pseudoisocytidine analog **2a** and its derivatives **2b** and **2c**.

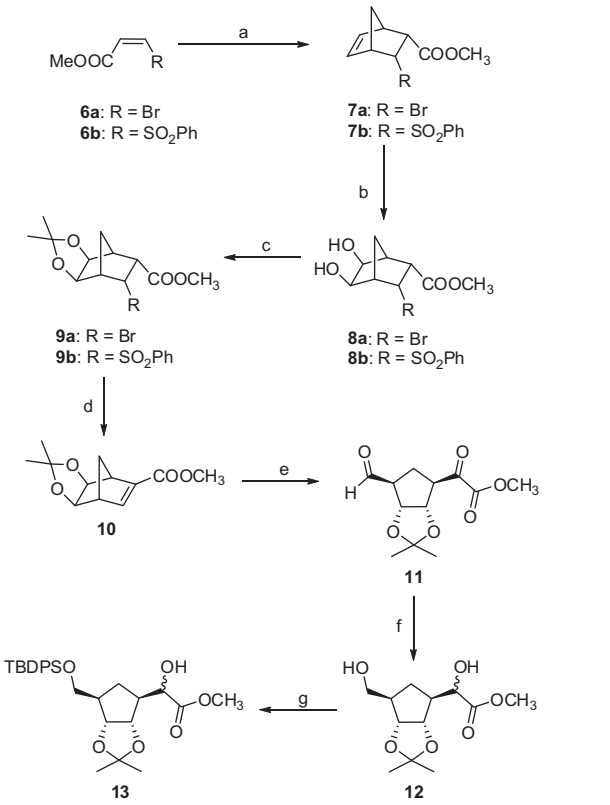
The overall retrosynthetic strategy is depicted in Scheme 2. It includes the previously described substituted norbornene intermediate **5**,[10] which could yield ketoester **4** after oxidative cleavage. The desired stereochemistry of **4** is dictated by the geometry of the bicyclo[2.2.1]heptene scaffold produced in a Diels–Alder reaction between cyclopentadiene and appropriately substituted dienophile **6**, possessing a leaving group that is utilized in a subsequent elimination-diastereoselective *cis*-dihydroxylation sequence. Compound **4** was envisioned as a precursor to the novel aldehyde-ester **3**, which upon reaction with urea, thiourea or guanidine, and global deprotection would provide the target compounds **2a–c**.

The synthesis started from commercially available methyl propiolate, which was converted into methyl (*Z*)-3-bromo-2-propenoate (**6a**) (Scheme 3).[11]

Since we have found that the bromo compound **6a** irritates the skin (especially upon repeated exposure), we have utilized an alternative starting material for the Diels–Alder reaction, sulfone **6b**.[12] Thermal Diels–Alder reaction between cyclopentadiene and **6a** was very sluggish—the conversion after 24 h in refluxing benzene or toluene was negligible and significant dimerization of cyclopentadiene occurred. On the other hand, the reaction proceeded



Scheme 2. Retrosynthetic analysis of pseudoisocytidine analogs (PG = protecting group).



Scheme 3. Reagents and conditions: (a) $EtAlCl_2$, cyclopentadiene, CH_2Cl_2, 0 °C; 80% for **7a**, 70–95% for **7b**. (b) OsO_4, NMO, acetone/H_2O (4:1), 40 °C; 80% for **8a**, 90% for **8b**. (c) $Me_2CH(OMe)_2$, cat. TsOH, acetone, rt; 99% for **9a** and **9b**. (d) DBU, Et_2O, 0 °C to rt; 95%, for **9a**, DBU, CH_3CN, 90 °C; 86% for **9b**. (e) O_3, CH_2Cl_2, −78 °C then Me_2S −78 °C to rt. (f) Li(Al-O-*t*Bu)$_3$H, THF, 0 °C to rt; 50–80% from **10**. (g) TBDPSCl, imidazole, CH_2Cl_2, rt; 70–92%.

smoothly at low temperature (0 °C), catalyzed by $EtAlCl_2$, with very good diastereoselectivity (9:1 *endo*/*exo*). Sulfone **6b** underwent an uncatalyzed Diels–Alder reaction quite efficiently (60% yield, rt, 14 h), although a higher conversion and yield were obtained in the presence of the catalyst ($EtAlCl_2$). The structure of the major *endo* diastereomer **7b** was confirmed by X-ray crystallography (see Supporting information). Diastereoselective *cis*-dihydroxylation of both adducts **7a** and **7b** provided the corresponding diols **8a** and **8b**, which were subsequently protected as acetonides. Elimination of the bromide or phenylsulfonyl group under basic conditions afforded the key intermediate **10**, which underwent ozonolytic cleavage to produce the rather unstable aldehyde **11** that was immediately used in the next step without purification. It should be noted that, in principle, compound **10** could be prepared more directly by dihydroxylation and protection of the Diels–Alder adduct of methyl propiolate and cyclopentadiene, which we prepared in 60–80% yield (cat. $AlCl_3$, PhH, 0 °C). Attempted dihydroxylations of the adduct, however, yielded complex hydroxylation mixtures that contained the desired product together with the unwanted diastereomer, the regioisomer with a dihydroxylated double bond in the vicinity of the ester group as well as a tetrahydroxylated product. In accordance with the published results,[10] our attempts to selectively reduce the aldehyde in the presence of an α-ketoester were not successful. On the other hand, reduction with excess Li(Al-O-*t*-Bu)$_3$H yielded an inseparable mixture of epimeric diols in which the primary hydroxyl group could be selectively silylated to provide α-hydroxyester **13** (Scheme 3).

Oxidation of the hydroxyl group in **13** proved challenging. Many standard methods (e.g., MnO_2, PDC, $KMnO_4$, Swern oxidation) including RuO_2 plus $NaIO_4$ conditions that were previously used

for a structurally similar intermediate,[10] failed to give the desired product **14** in acceptable yield. Fortunately, oxidation with Dess–Martin periodinane yielded pure α-ketoester **14** in very good yield and purity (Scheme 4).

One-carbon homologation was accomplished via the Wittig reaction with methoxymethylenetriphenylphosphorane. The reaction produced enol ether **15** in 37–65% yield as a separable mixture of *Z* and *E* isomers (*Z*:*E* ~7:5). It should be noted that the Wittig olefination was rather sensitive to the type and quality of base. Generation of the phosphonium ylide with LDA gave the most consistent and reproducible results, while reactions with LiHMDS, KHMDS, or *t*-BuOK afforded olefination products in substantially lower yields. Attempts to hydrolyze selectively enol ether **15** into the desired aldehyde **3** in the presence of the acetonide and TBPDS groups met with only limited success. With PPTS or acetic acid, partial cleavage of the acetonide and/or TBDPS groups was observed, while the enol ether moiety remained intact. We thus attempted direct transformation of **15** into pyrimidine **16b** by reaction with urea. With sodium ethoxide in ethanol or NaH in THF, we observed mainly cleavage of the TBDPS group and the desired product **16b** was formed in very low yield. However, using *t*-BuOK in *t*-BuOH as the base, the desired product was formed in good yield. Reactions of **15** with thiourea and guanidine under similar conditions yielded compounds **16c** and **16a**, respectively. Selective deprotection of the TBDPS group in pyrimidines **16a**–**c** with TBAF revealed the primary hydroxyl group, which could be utilized for further selective derivatization, for example, the preparation of phosphates and its isosteres. Final hydrolysis of the acetonide under acidic conditions provided the target compounds **2a**, **2b,** and **2c** in good overall yields. The relative configurations of **2a**–**c** were confirmed by 2D NMR experiments (shown in Supporting information). We were able to separate the enantiomers of intermediates **17b** and **17c** as well as **16a** by HPLC on a chiral stationary phase (see Supporting information).

Clearly, the strategy described above can only be applied to construct the (hetero)cyclic bases by elaboration of the ketoester **14.** In order to target compounds that are unaccessible by the methodology described above, we envisioned a different and perhaps more general route that utilizes a versatile cyclopentanone intermediate (**19**, Scheme 5).

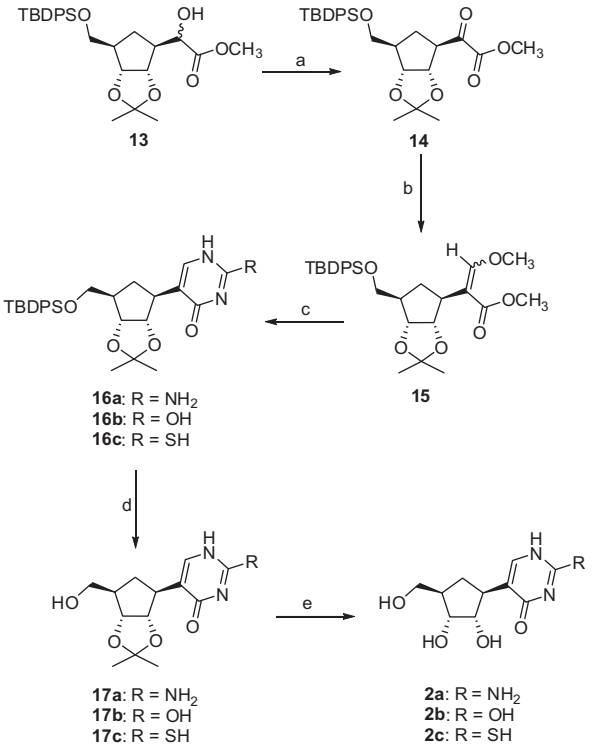
In order to access quickly and evaluate the potential of compound **19**, we converted one of the synthetic intermediates, ketoester **14**, into the corresponding silyl enol ether **18**. Subsequent ozonolysis of this, rather unusual,[13] substrate afforded **19** in an acceptable yield (Scheme 5). The two-step sequence provided sufficient amounts of material for preliminary studies. We initially studied the introduction of a phenyl group using PhMgBr or PhLi under a variety of conditions (e.g., variable temperature and solvent, presence or absence of $CeCl_3$) and found that the best results were obtained when PhLi was added to a solution of **19** in THF at 0 °C. Under these conditions, a single diastereomer of addition product **20**, resulting from attack of the reagent from the less sterically hindered side of **19**, was obtained in 75% yield (Scheme 6). We were unable to detect the other diastereomer by NMR spectroscopy.

The relative stereochemistry of the addition product **20** (supported by 2D NMR; see Supporting information) was unambiguously assigned by X-ray crystallography of its *p*-bromobenzoyl derivative **24** (Scheme 6 and Fig. 2).

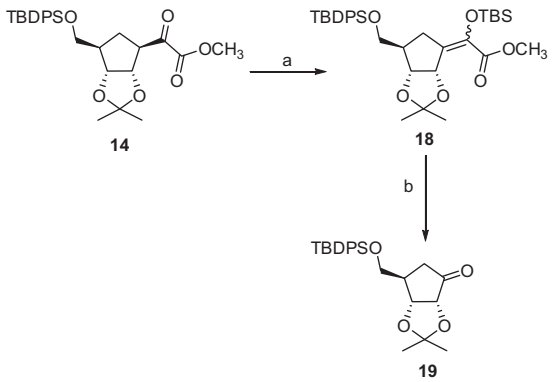
We tested the effect of compounds **2a**, **2b,** and **2c** on the viability of leukemia cell lines that were available to us: SU-DHL-4 (diffuse large B-cell lymphoma, del/mut TP53), JEKO-1 (mantle cell lymphoma, del/mut TP53), and JVM-3 (mantle cell lymphoma, wt-TP53). The viability of SU-DHL-4 and JEKO-1 was not affected by 10 μM or 100 μM concentrations of the compounds. However, JVM-3 was more sensitive: 90% viability was observed upon treatment with 100 μM **2c**; with 10 μM and 100 μM **2a** we observed 90% and 83% viability, respectively.

Since the arrangement around the tertiary alcohol carbon of compound **22** mimics that of the acetal product of glycosylase-mediated cleavage,[14] this compound was tested against glycosylases NEIL1, NEIL2, NTH1, and hOGG1, and was found to inhibit selectively NEIL1 in a dose-dependent manner: at 1 mM we observed 46% inhibition, and at 0.5 mM and 0.125 mM concentrations 22% and 2% inhibition, respectively.

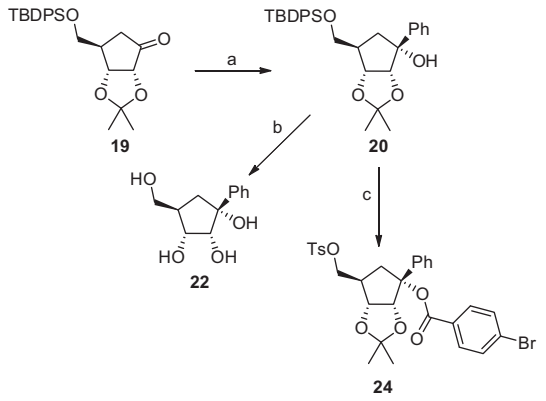
In summary, we have completed the first syntheses of three new racemic carbocyclic nucleoside analogs (**2a**–**c**) of pseudoisocytidine, each in 13 steps. The synthetic approach builds on a user-friendly preparation of sulfone **7b**, which can be diastereoselectively dihydroxylated and ultimately elaborated into tetrasubstituted chiral cyclopentanes **11** with good diastereoselectivity. While



Scheme 4. Reagents and conditions: (a) Dess–Martin periodinane, CH_2Cl_2, 0 °C to rt; 80–90%. (b) $Ph_3P^+CH_2OMeCl^-$, LDA, THF, 0 °C to rt; 37–65% (*Z*:*E* 7:5). (c) guanidinium·HCl for compound **16a** (20–35%), urea for **16b** (30–45%) and thiourea for **16c** (40–50%), *t*-BuOK, *t*-BuOH, reflux. (d) TBAF, wet THF, rt; 90% for **17a**, 96% for **17b**, 90% for **17c**. (e) HCl/H_2O/MeOH 1:1:1, rt; 69% for **17a**, 76% for **17b**, 71% for **17c**.



Scheme 5. Reagents and conditions: (a) LiHMDS, TBSOTf, THF, −78 °C. (b) O_3, CH_2Cl_2, −78 °C, then Me_2S −78 °C to rt; 52% from **14**.



Scheme 6. Reagents and conditions: (a) PhLi, THF, 0 °C; 75%; (b) TBAF, wet THF, rt; then PPTS, MeOH rt; 42% from **20**. (c) TBAF, wet THF, rt, then TsCl, Et_3N, DMAP, CH_2Cl_2, 0 °C; then NaH, 4-BrC_6H_4COCl, THF, 0 °C to rt, 42% from **20**.

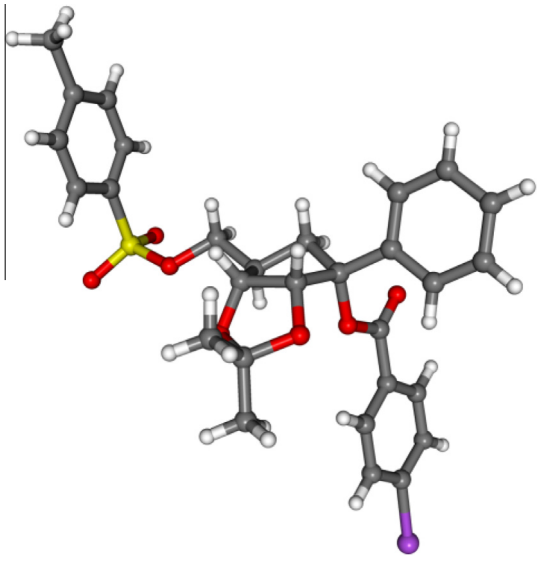


Figure 2. X-ray crystal structure of compound **24** (CCDC Ref. No. 937690).

Diels–Alder reactions between cyclopentadiene with a beta-sulfonyl enoate are known,[15] the strategic elaboration of readily accessed sulfones **7b** into cyclopentanes seems largely undeveloped, and can be used for the construction of nucleoside analogs and for target directed syntheses. Analogs of α-ketoester intermediate **14** have been previously used for the construction of the heterocyclic ring directly,[6d] or after a two carbon homologation;[6f] our one-carbon homologation enables the synthesis of additional (hetero)cycles. Furthermore, we have performed preliminary studies toward a potentially more versatile strategy for the preparation of carbocyclic nucleoside analogs that utilizes highly diastereoselective additions of organometallic reagents onto cyclopentanone **19**, which itself is available in two-steps from α-ketoester intermediate **14**. While analogs of **19** are known and have been used in synthesis of carbocyclic nucleosides,[16] tetraol **22**, to our knowledge, is the only carbocyclic C-nucleoside represented by generic structure **D**, where R is an oxygenated substituent.

Unlike the analogs in the series **A**, **B**, and **C** (Fig. 1), compounds such as **22** are stable and their biological evaluation should enable mapping of part of the chemical space that is currently unaccessible. Along this line, we have tested the prepared carbocyclic analogs in three leukemia cell lines, and compounds **2a** and **2c** were found to be moderately active against wt-TP53—mantle cell lymphoma cell line JVM-3, which is generally the most sensitive to chemotherapeutic treatment. The ability of compound **22** to inhibit glycosylase NEIL1 has served as the starting point for a more thorough exploration of the biological activity of this series of novel carbocyclic nucleoside analogs. Further studies are currently in progress and the results will be published elsewhere.

Acknowledgments

This project was funded from the SoMoPro programme (SRGA771). Research leading to these results has received a financial contribution from the European Community within the Seventh Framework Programme (FP/2007-2013) under Grant Agreement No. 229603. The research was also co-financed by the South Moravian Region. The authors would like to thank Dr. Jakub Švenda for helpful comments and a review of the manuscript.

Supplementary data

Supplementary data (experimental procedures, spectral characterization, and copies of ^1H and ^{13}C NMR data) associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.tetlet.2014.05.030.

References and notes

1. *Modified Nucleosides in Biochemistry, Biotechnology and Medicine*; Herdewijn, P., Ed.; Wiley-VCH, 2008.
2. (a) Agrofoglio, L.; Suhas, E.; Farese, A.; Condom, R.; Challand, R. S.; Earl, R. A.; Guedj, R. *Tetrahedron* **1994**, *50*, 10611; (b) Hildbrand, S.; Troxler, T.; Scheffold, R. *Helv. Chim. Acta* **1994**, *77*, 1236; (c) Altmann, K. H.; Kesselring, R. *Synlett* **1994**, 853; (d) Crimmins, M. T. *Tetrahedron* **1998**, *54*, 9229; (e) Zhu, X.-F. *Nucleosides, Nucleotides, Nucleic Acids* **2000**, *19*, 651; (f) Agrofoglio, L. A. *Curr. Org. Chem.* **2006**, *10*, 333.
3. (a) Borchardt, R. T.; Wu, Y. S.; Huber, J. A.; Wycpalek, A. F. *J. Med. Chem.* **1976**, *19*, 1104; (b) Secrist, J. A.; Clayton, S. J.; Montgomery, J. A. *J. Med. Chem.* **1984**, *27*, 534; (c) Neres, J.; Labello, N. P.; Somu, R. V.; Boshoff, H. I.; Wilson, D. J.; Vannada, J.; Chen, L.; Barry, C. E.; Bennett, E. M.; Aldrich, C. A. *J. Med. Chem.* **2008**, *51*, 5349; (d) Choi, W. J.; Chung, H.; Chandra, G.; Alexander, V.; Zhao, L. X.; Lee, H. W.; Nayak, A.; Majik, M. S.; Kim, H. O.; Kim, J.-H.; Lee, Y. B.; Ahn, C. H.; Lee, S. K.; Jeong, L. S. *J. Med. Chem.* **2012**, *55*, 4521.
4. (a) Arita, M.; Adachi, K.; Ito, Y.; Sawai, H.; Ohno, M. *J. Am. Chem. Soc.* **1983**, *105*, 4049; (b) Arai, Y.; Hayashi, Y.; Yamamoto, M.; Takayema, H.; Koizumi, T. *J. Chem. Soc., Perkin Trans. 1* **1988**, 3133.
5. (a) Popsavin, M.; Spaic, S.; Svirčev, M.; Kojic, V.; Bogdanovic, G.; Popsavin, V. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 5317; (b) Štambaský, J.; Hocek, M.; Kočovský, P. *Chem. Rev.* **2009**, *109*, 6729; (c) Bárta, J.; Slavětínská, L.; Klepetářová, B.; Hocek, M. *Eur. J. Org. Chem.* **2010**, 5432.
6. (a) Playtis, A. J.; Fissekis, J. D. *J. Org. Chem.* **1975**, *40*, 2488; (b) Chu, C. K. I.; Wempen, I.; Watanabe, K. A.; Fox, J. J. *J. Org. Chem.* **1976**, *41*, 2793; (c) Just, G.; Kim, S. *Tetrahedron Lett.* **1976**, *14*, 1063; (d) Just, G.; Kim, S. *Can. J. Chem.* **1977**, *55*, 427; (e) Saksena, A. K.; Ganguly, A. K. *Tetrahedron Lett.* **1981**, *22*, 5227; (f) Takahashi, T.; Kotsubo, H.; Koizumi, T. *Tetrahedron: Asymmetry* **1991**, *2*, 1035; (g) Dishington, A. P.; Humber, D. C.; Stoodley, R. J. *J. Chem. Soc., Perkin Trans. 1* **1993**, 57; (h) Díaz, M.; Ortuño, R. M. *Tetrahedron: Asymmetry* **1997**, *8*, 3421.
7. Burchenal, J. H.; Ciovacco, K.; Kalaher, K.; O'Toole, T.; Kiefner, R.; Dowling, M. D.; Chu, C. K.; Watanabe, K. A.; Wempen, I.; Fox, J. J. *Cancer Res.* **1976**, *36*, 1520.
8. Woodcock, T. M.; Chou, T. C.; Tan, C. T. C.; Sternberg, S. S.; Philips, F. S.; Young, C. W.; Burchenal, J. H. *Cancer Res.* **1980**, *40*, 4243.
9. Grierson, J. R.; Shields, A. F.; Zheng, M.; Kozawa, S. M.; Courter, J. H. *Nucl. Med. Biol.* **1995**, *22*, 671.
10. (a) Just, G.; Reader, G. *Tetrahedron Lett.* **1973**, *17*, 1521; (b) Just, G.; Reader, G.; Faure, B. C. *Can. J. Chem.* **1975**, *54*, 849.
11. (a) Ma, S.; Lu, X. *Org. Synth.* **1999**, *9*, 415; (b) Rossi, R.; Bellina, F.; Catanese, A.; Mannina, L.; Valensin, D. *Tetrahedron* **2000**, *56*, 479.
12. Hirst, G. C.; Parsons, P. J. *Org. Synth.* **1990**, *69*, 169.
13. We have been unable to find any examples of the ozonolysis of such tetrasubstituted alkenes.
14. (a) Slupphaug, G.; Mol, C. D.; Kavli, B.; Arvai, A. S.; Krokan, H. E.; Tainer, J. A. *Nature* **1996**, *384*, 87; (b) Parikh, S. S.; Mol, C. D.; Slupphaug, G.; Bharati, S.; Krokan, H. E.; Tainer, J. A. *EMBO J.* **1998**, *17*, 5214.
15. Downey, C. W.; Craciun, S.; Vivelo, C. A.; Neferu, A. M.; Mueller, C. J.; Corsi, S. *Tetrahedron Lett.* **2012**, *53*, 5766.
16. Tanaka, M.; Yoshioka, M.; Sakai, K. *Tetrahedron: Asymmetry* **1993**, *4*, 981.