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Studies on the porcine liver esterase-catalyzed hydrolysis of pentaacetyl catechin and epicatechin: Application to the synthesis of novel dimers and trimers

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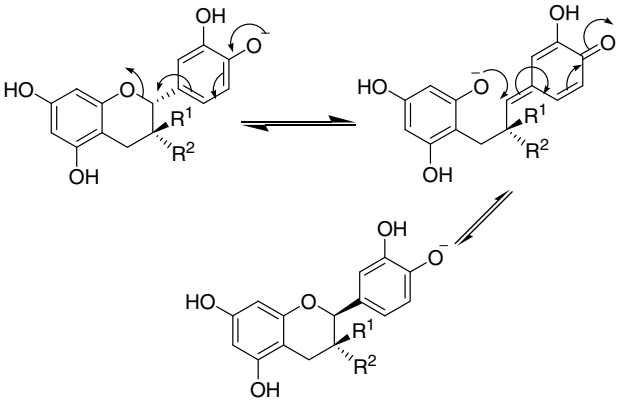
ABSTRACT

Porcine liver esterase-catalyzed hydrolysis of 3,5,7,3′,4′-pentaacetylated catechin was studied. The selectivity of the enzyme in hydrolyzing the acetate moiety is time dependent. Careful control of the duration of hydrolysis makes it possible to isolate the differentially protected catechins. Similar result was also obtained in the epicatechin series. These results are important for elaboration of epicatechin or catechin into different derivatives with defined regiochemistry. These include novel dimeric and trimeric architectures.

The diverse array of pharmacological properties[1] as well as the continuing industrial use[2] is the main reasons why procyanidin oligomers have had considerable attention of scientists in recent years. As a result, efforts are ongoing to synthesize structures with more than one monomer unit (catechin or epicatechin), both natural and designed ones. We encountered a problem of deacetylation of per acetylated catechin or epicatechin or their oligomers by hydrolysis while attempting to develop[3] a general method for their synthesis in a region and in a stereocontrolled manner.[4–6] While deacetylation of phenolic esters is a simple base-mediated hydrolysis, the situation, in these systems, is much more complicated in flavonoid systems. The stereochemical integrity at C-2 is lost[7] in the process, as shown in Scheme 1. Moreover, for regiospecific coupling, one needs differentially protected catechin or epicatechin derivatives. The free phenolic hydroxyl group acts as an activator of the aromatic ring to which it is attached. Earlier we have reported the preparation of 3,7,3′,4′-tetraacetoxy catechin/epicatechin by a Porcine Liver Esterase (PLE)-mediated hydrolysis of pentaacetylated monomers.[8] In this account, we describe the isolation of differentially acetylated catechin/epicatechin. We have shown that by controlling the time of hydrolysis, tetra and diacetates can be obtained in decent yields and in regiochemically pure forms. Only the triacetates were obtained as a mixture of regioisomers. Isolation of these partially protected catechin/epicatechin derivatives will help to synthesize higher oligomers in a regiocontrolled manner preserving their stereochemical integrity, which is a great challenge to the synthetic chemist.

In our earlier work,[8] we had shown that PLE-catalyzed hydrolysis of pentaacetyl catechin or epicatechin led to 3,7,3′,4′-tetraacetyl derivatives or 3-acetyl derivatives depending upon the duration of hydrolysis (1 or 24 h, respectively) (Scheme 2). Moreover, the



Scheme 1. Base-catalyzed epimerization.

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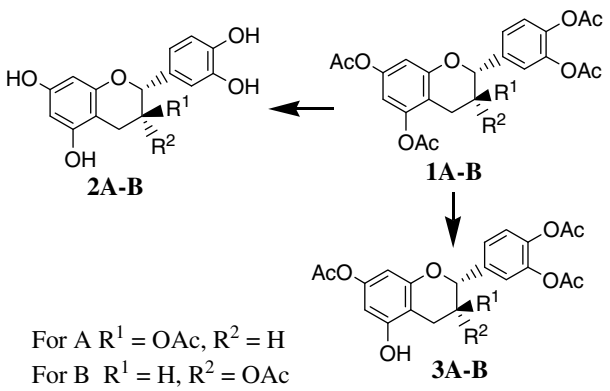


Scheme 2. PLE-catalyzed hydrolysis for short and long duration.

hydrolysis was shown to be free from any epimerization. The specific question that we wanted to address is whether the phenolic acetates are hydrolyzed in a sequential manner and whether it is possible to isolate products corresponding to di or tri acetates if the hydrolysis is carried out for intermediate duration. For this, we needed to monitor the reaction at different time points.

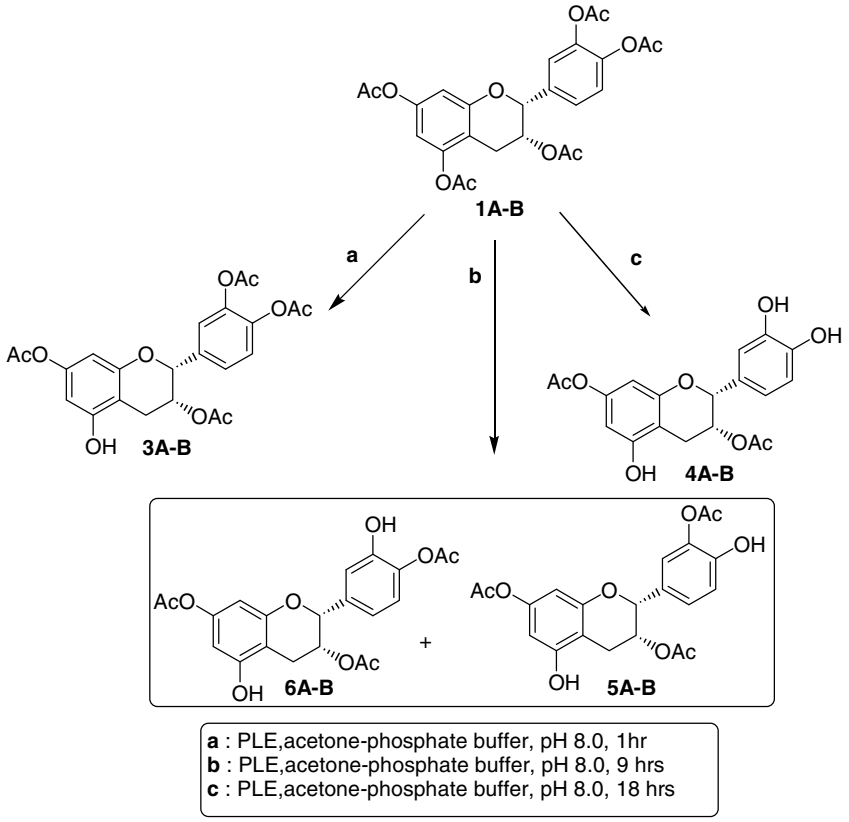
When the reaction was allowed to proceed up to 9 h, a mixture of triacetoxy compounds was isolated. The same mixture of compounds was also isolated if the tetraacetoxy isomer was hydrolyzed separately for 8 h. The upfield shift of ring C-protons indicated that the hydrolysis had taken place either at C-4′ or C-3′. One of the compounds was 3,7,3′-triacaetoxy catechin/epicatechin, while the other was 3,7,4′-triacetoxy isomer. Unfortunately, these compounds could not be separated even by HPLC. When the hydrolysis was allowed to proceed up to 18 h, only one diacetoxy compound was isolated (Scheme 3) whose structure was assigned as the 3, 7-diacetoxy catechin/epicatechin on the basis of NMR studies. The upfield shift of both 2′- and 5′-protons pointed out the hydrolysis of 3′ and 4′-acetoxy groups. No shift was observed for the protons in ring A. All these results are summarized in Table 1. The formation of similar hydrolysis products from both catechin and epicatechin acetates ruled out the influence of C-3 acetate on the course of hydrolysis.

One application of this highly regioselective hydrolysis is manifested in the synthesis of novel catechin and epicatechin-based dimeric and trimeric molecules. Thus the tetraacetoxy catechin, obtained from the corresponding pentaacetate by regioselective PLE-catalyzed hydrolysis as described, when treated with mesitoyl chloride (0.34 equiv) and triethyl amine (0.34 equiv) in methylene

Table 1
Result of PLE-catalyzed hydrolysis

Duration of hydrolysis (h)	Major product	Isolated yield (%)	Ratio[a] of penta:tetra:tri:di:mono acetates
1	Tetraacetoxy catechin/epicatechin	70	1:5:1:nd:nd[a]
9	Triacetoxy catechin/ epicatechin	48	1:5:10:2:nd
18	Diacetoxy catechin/ epicatechin	50	nd:nd:6:10:2
24	3-Acetoxy catechin/ epicatechin	97	Mostly nono

[a] Ratio determined by HPLC (ODS column, 15% H_2O in MeOH as the mobile phase; nd means not determined.



Scheme 3. Time dependent PLE-catalyzed hydrolysis.

chloride, furnished the novel C_3-symmetric trimer **7A** in 85% yield as a white solid (Scheme 4). The structure of **7A** was unequivocally established by thorough analysis of ^1H, ^{13}C NMR, and mass (MALDI) spectral data. In a similar way, the epicatechin acetate-based trimer **7B** has also been prepared and characterized. In order to explore the possibility whether these trimers can act as dendrimeric core, **7A** and **7B** were subjected to PLE-catalyzed hydrolysis. However, the hydrolysis led to a complex mixture of products demonstrating poor selectivity.

The various dimeric catechin or epicatechins **8A–10A** and **8B–10B** were prepared by treating 2 mol equivalent of the tetraacetate with 1 mol equivalent of phthaloyl/isophthaloyl or terephthaloyl chloride. The linked dimers were obtained in 90% yield after Silica gel column chromatography.

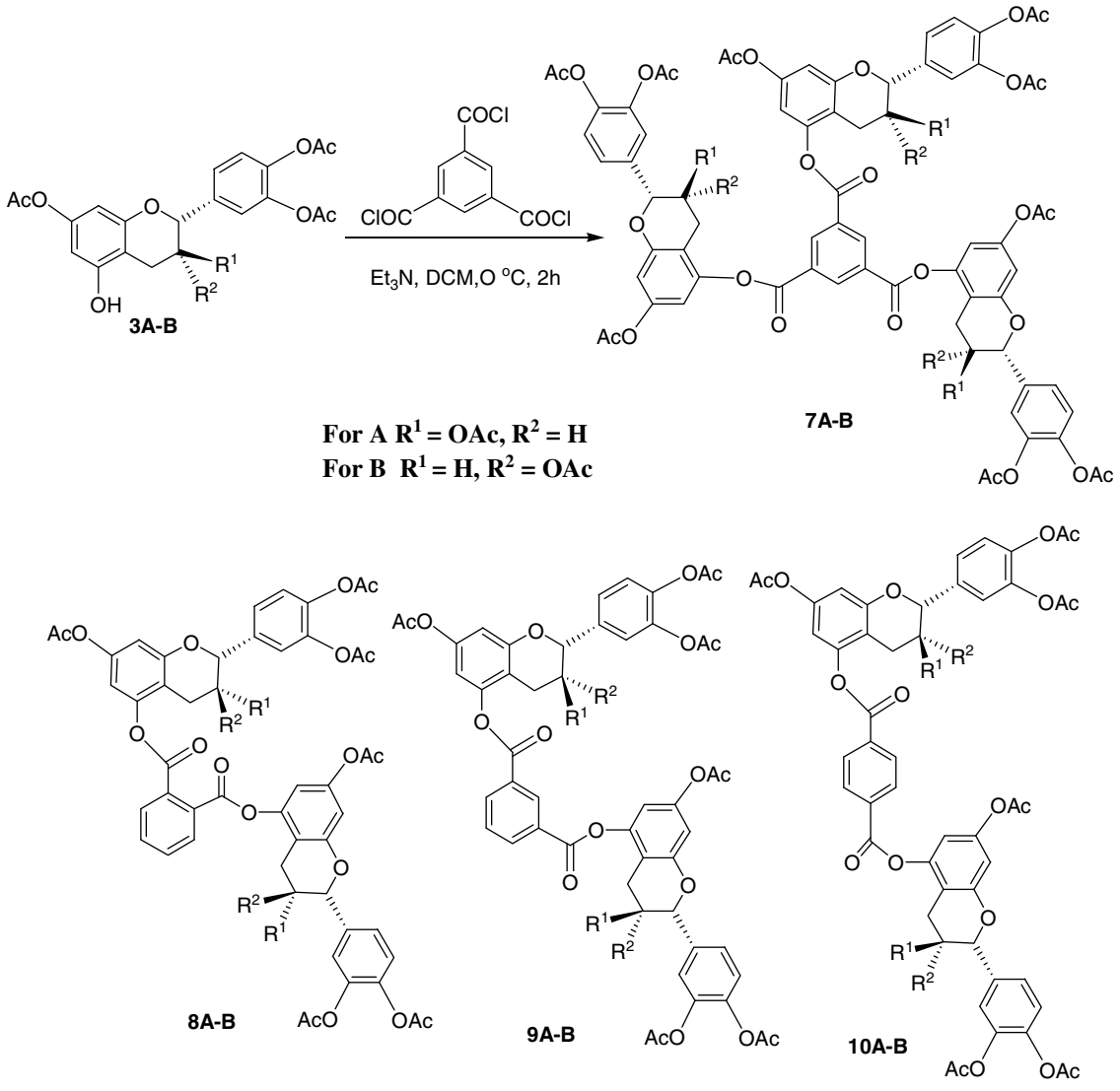
In conclusion, we have developed an enzymatic method for the sequential deprotection of phenolic acetates in catechin or epicatechin series in regiospecific manner without any loss of stereochemical integrity. The synthesis of novel catechin and epicatechin acetate-based dimeric and trimeric systems demonstrated the utility of corresponding partially acetylated derivatives. Currently, we are studying the antioxidant activity of these novel polyphenols and the results will be reported in due course.

Selected experimental procedure and spectral data. *General hydrolysis procedure:* 3,5,7,3′,4′-Pentaacetyl catechin (**1**) (200 mg, 0.4 mmol) was dissolved in acetone (10 ml). Phosphate buffer (pH 8.0, 20 ml) was added followed by PLE as crude acetone powder[9] (100 mg) and the mixture was stirred at room temperature for 1–24 h. It was then filtered through celite and the filtrate was extracted with ethyl acetate. The organic layer was dried, filtered, and evaporated to leave a brownish solid from which the major products were purified by Si-gel chromatography.

*3,7,3′-Triacetyl (+) catechin and 3,7,4′-triacetyl (+) catechin (mixture) (**5A** and **6A**):* δ_H (200 MHz, $CDCl_3$) 6.79–7.05 (3H, m, H-2′, 5′, 6′), 6.29 (1H, d, J = 1.7 Hz, H-8), 6.14 (1H, d, J = 2.0 Hz, H-6), 6.10 (1H, d, J = 2.1 Hz, H-6), 5.25 (1H, t, J = 5.0 Hz, H-3), 5.15 (1H, d, J = 4.7 Hz, H-2), 5.08 (1H, d, J = 5.4 Hz, H-2), 2.44–2.60 (2H, m, H-4), 2.38 (3H, s, 3′-OAc or 4′-OAc), 2.27 (3H, s, 7-OAc), 2.0 (3H, s, 3-OAc), 1.98 (3H, s, 3-OAc); Mass (ESI) 416 (M^+), 374, 355, 313.

*3,7-Diacetyl (+) catechin (**4A**):* δ_H (200 MHz, $CDCl_3$) 6.79–6.81 (2H, m, H-2′, 6′), 6.69–6.74 (1H, m, H-5′), 6.29 (1H, d, J = 2.0 Hz, H-8), 6.09 (1H, d, J = 2.2 Hz, H-6), 5.26 (1H, t, J = 5.3 Hz, H-3), 5.09 (1H, d, J = 5.2 Hz, H-2), 2.30–2.57 (2H, m, H-4), 2.28 (3H, s, 7-OAc), 2.0 (3H, s, 3-OAc); Mass (ESI) 374 (M^+), 355, 313.

*3,7,3′-Triacetyl (−) epicatechin(**10**) and 3,7,4′-triacetyl (−) epicatechin (**11**) (mixture) (**5B** and **6B**):* δ_H (200 MHz, $CDCl_3$) 6.79–7.11



Scheme 4. Synthesis of dimeric and trimeric architecture.

(3H, m, H-2′, 5′, 6′), 6.31 (1H, bs, H-8), 6.17 (1H, bs, Hz, H-6), 5.39 (1H, bs, H-3), 4.95 (1H, bs, H-2), 2.91 (2H, bs, H-4), 2.43 (3H, s, 3′-OAc or 4′-OAc), 2.27 (3H, s, 7-OAc), 1.89 (3H, s, 3-OAc), 1.80 (3H, s, 3-OAc); Mass (ESI) 416 (M^+), 374, 355.

*3,7-Diacetyl (−) epicatechin (**4B**):* δ_H (200 MHz, $CDCl_3$) 6.95 (1H, s, H-5′), 6.83 (1H, s, H-2′, 6′), 6.33 (1H, d, J = 2.02 Hz, H-8), 6.19 (1H, d, J = 2.16 Hz, H-6), 5.42 (1H, bs, H-3), 4.97 (1H, s, H-2), 2.97 (2H, m, H-4), 2.28 (3H, s, 7-OAc), 1.92 (3H, s, 3-OAc); Mass (ESI) 374 (M^+).

General method for the synthesis of dimers and trimers: To a solution of tetraacetyl catechin/epicatechin (1 equiv) in CH_2Cl_2 (15 mL), mesitoyl chloride (0.34 equiv) or phthaloyl/isophthaloyl/terepthaloyl chloride (0.5 equiv) was added followed by Et_3N (0.34 equiv or 0.5 equiv, respectively). The mixture was stirred for 2 h at 0 °C. It was then poured into water and extracted with CH_2Cl_2. The organic layer was dried and evaporated. The residue upon Si-gel chromatography (hexane/EA 2:1) furnished the trimers or dimers as white solids (85–90%).

*Benzene-1,3,5-tricarboxylic acid tris-[3,7-diacetoxy-2-(3,4-diacetoxy-phenyl)-chroman-5-yl] ester (catechin) (**7A**):* δ_H (200 MHz, $CDCl_3$) 9.13 (3H, s, Ar-H), 7.29–7.17 (3H, m, H-6′, 5′, 2′) 6.72 (2H, ABq, J = 1.8 Hz, H-8, 6), 5.29–5.16 (2H, m, H-3, 2), 2.89–2.75 (2H, m, H-4), 2.27 (9H, s, 7, 3′, 4′-OAc), 1.96 (3H, s, 3-OAc); δ_C (50 MHz, $CDCl_3$) 170.04, 168.83, 167.99, 162.06, 154.59, 150.05, 149.14, 142.07, 136.33, 135.97, 130.64, 124.38, 123.70, 121.78, 110.48, 108.76, 108.32, 68.07, 24.07, 21.06, 20.89, 20.59; Mass (MALDI) 1548.1447 (M^+ +18).

*Benzene-1,3,5-tricarboxylic acid tris-[3,7-diacetoxy-2-(3,4-diacetoxy-phenyl)-chroman-5-yl] ester (epicatechin) (**7B**):* δ_H (200 MHz, $CDCl_3$) 9.18 (3H, s, Ar-H), 7.38 (1H, s, H-2′), 7.25 (2H, Abq, J = 8.2 Hz, H-6′, 5′) 6.75 (2H, Abq, J = 2.2 Hz, H-8, 6), 5.43 (1H, bs, H-3), 5.17 (1H, bs, H-2), 3.09–2.98 (2H, m, H-4), 2.30 (9H, s, 7, 3′, 4′-Oac), 1.99 (3H, s, 3-Oac); δ_C (50 MHz, $CDCl_3$) 170.32, 168.92, 168.03, 168, 162.08, 155.19, 149.43, 142, 141.89, 136.37, 135.72, 130.68, 124.23, 122, 109.8, 108.77, 66.46, 26.18, 21.04, 20.76, 20.59; Mass (MALDI) 1548.1225 (M^+ +18).

*Phthalic acid bis-[3,7-diacetoxy-2-(3,4-diacetoxy-phenyl)-chroman-5-yl] ester (catechin) (**8A**):* δ_H (400 MHz, $CDCl_3$) 8.03 (2H, m), 7.69 (2H, m), 7.27 (6H, m), 6.74 (2H, bs) 6.70 (2H, bs), 5.26 (2H, m), 5.14 (2H, d, J = 6.4 Hz), 2.94 (2H,dd, J = 5.2, 16.8 Hz), 2.74 (2H, d, J = 16.8 Hz), 2.28 (s, 18H), 1.94 (s, 6H); δ_C (100 MHz, $CDCl_3$) 170.0, 169.0, 168.1, 164.5, 154.4, 149.9, 149.2, 142.0, 141.9, 136.0, 135.9, 132.1, 131.0, 129.5, 125.7, 124.5, 123.6, 121.8, 110.4, 108.8, 108.1, 77.6, 68.1, 24.0, 21.1, 20.8, 20.6; Mass (ESI) 1047 (MH^+); HRMS Calcd for $C_{54}H_{46}O_{22}+H^+$ 1047.2544. Found 1047.2547.

*Phthalic acid bis-[3,7-diacetoxy-2-(3,4-diacetoxy-phenyl)-chroman-5-yl] ester (epicatechin) (**8B**):* δ_H (400 MHz, $CDCl_3$) 8.02 (2H, m), 7.65 (2H, m), 7.37–7.19 (6H, m), 6.74 (2H, d, J = 4.4 Hz,) 6.70 (2H, d, J = 4.4 Hz), 5.36 (2H, bs), 5.18 (2H, s), 3.06 (2H,dd, J = 4.4, 18.0 Hz), 2.50 (2H, d, J = 18.0 Hz), 2.29 (12H, s), 2.26 (6H, s), 1.89 (6H, s,); δ_C (100 MHz, $CDCl_3$) 170.4, 169.01 168.1, 168.0, 164.7, 155.1, 149.7, 149.5, 141.9, 141.8, 136.0, 135.9, 132.2, 131.2, 131.0, 130.1, 129.5, 129.0, 124.4, 123.2, 122.0, 110.0, 108.8, 108.4, 77.6, 66.5, 26.0, 21.0, 20.7, 20.6; Mass (ESI) 1047 (MH^+), 1069 (MNa^+).

*Isophthalic acid bis-[3,7-diacetoxy-2-(3,4-diacetoxy-phenyl)-chroman-5-yl] ester (catechin) (**9A**):* δ_H (400 MHz, $CDCl_3$) 8.91 (1H, s), 8.41 (2H, d, J = 8.0 Hz), 7.68 (1H, t, J = 7.6 Hz), 7.28–7.18 (6H, m), 6.73 (4H, s) 6.70), 5.26 (2H, m), 5.18 (2H, d, J = 6.0 Hz), 2.92 (2H, dd, J = 5.2, 16.8 Hz), 2.73 (2H, dd, J = 6.4, 16.8 Hz), 2.29 (12H, s), 2.28 (6H, s), 2.02 (6H, s); δ_C (100 MHz, $CDCl_3$) 170.0, 169.0, 168.1, 164.5, 154.4, 149.9, 149.2, 142.0, 141.9, 136.0, 135.9, 132.1, 131.0, 129.5, 125.7, 124.5, 123.6, 121.8, 110.4, 108.8, 108.1, 77.6, 68.1, 24.0, 21.0, 20.8, 20.5; Mass (ESI) 1047 (MH^+); HRMS Calcd for $C_{54}H_{46}O_{22}+H^+$ 1047.2544. Found 1047.2540.

*Isophthalic acid bis-[3,7-diacetoxy-2-(3,4-diacetoxy-phenyl)-chroman-5-yl] ester (epicatechin) (**9B**):* δ_H (400 MHz, $CDCl_3$) 8.94 (1H, s), 8.44 (2H, dd, J = 1.6, 8.0 Hz), 7.71 (1H, t, J = 7.6 Hz), 7.29–7.19 (6H, m), 6.73 (4H, s), 5.41 (2H, bs), 5.16 (2H, s), 3.09 (2H, dd, J = 4.4, 18.0 Hz), 2.94 (2H, d, J = 18.0 Hz), 2.30 (18H, s), 1.92 (6H, s); δ_C (100 MHz, $CDCl_3$) 170.5, 169.0, 168.1, 163.2, 155.2, 149.8, 149.0, 142.0, 141.9, 136.0, 135.2, 132.0, 129.3, 129.1, 124.5, 123.3, 121.9, 110.0, 108.9, 108.5, 76.7, 66.8, 26.2, 21.0, 20.8, 20.5; Mass (ESI) 1047 (MH^+), 1069 (MNa^+).

*Terephthalic acid bis-[3,7-diacetoxy-2-(3,4-diacetoxy-phenyl)-chroman-5-yl] ester (catechin) (**10A**):* δ_H (400 MHz, $CDCl_3$) 8.28 (4H, s), 7.28 (2H, bs), 7.18 (4H, m), 6.75 (2H, s) 6.73 (2H, s), 5.29 (2H, m), 5.20 (2H, m), 2.92 (2H, dd, J = 4.8, 17.6 Hz), 2.71 (2H, dd, J = 6.0, 17.6 Hz), 2.30 (18H, s), 2.28, 2.0 (6H, s); δ_C (100 MHz, $CDCl_3$) 170.1, 169.0, 168.1, 163.0, 154.4, 149.9, 149.2, 142.0, 136.0, 133.3, 130.4, 124.3, 123.7, 121.7, 110.2, 108.8, 108.0, 77.5, 68.0, 23.8, 21.1, 21.0, 20.9; Mass (ESI) 1047 (MH^+), 1069 (MNa^+).

*Terephthalic acid bis-[3,7-diacetoxy-2-(3,4-diacetoxy-phenyl)-chroman-5-yl] ester (epicatechin) (**10B**):* δ_H (400 MHz, $CDCl_3$) 8.31 (4H, s), 7.37 (2H, d, J = 1.6 Hz), 7.27 (2H, dd, J = 1.6, 8.4 Hz), 7.21 (2H, d, J = 8.4 Hz, 6.75 (4H, s), 6.75 (2H, s), 5.41 (2H, bs), 5.20 (2H, s), 3.05 (2H, dd, J = 4.4, 17.2 Hz), 2.94 (2H, d, J = 17.2 Hz), 2.30 (18H, s), 1.93 (6H, s); δ_C (100 MHz, $CDCl_3$) 170.3, 169.0, 168.1, 168.0, 163.1, 155.1, 149.8, 149.6, 142.0, 141.9, 135.7, 133.3, 130.4, 124.3, 123.2, 122.0, 109.7, 108.8, 108.4, 77.2, 66.5, 26.1, 21.0, 20.7, 20.6; Mass (ESI) 1047 (MH^+), 1069 (MNa^+); HRMS Calcd for $C_{54}H_{46}O_{22}+H^+$ 1047.2544. Found 1047.2549.

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References and notes

1. (a) Haslam, E. In *The Flavonoid*; Harborne, J. B., Mabry, T. J. H., Eds.; Academic Press: New York, 1975; pp 505–559; (b) Haslam, E. In *The Flavonoids. Advances in Research*; Harborne, J. B., Ed.; Chapman and Hall: London, 1982; pp 417–447; (c) Geiger, H.; Quinn, C. In *The Flavonoids. Advances in Research*; Harborne, J. B., Ed.; Chapman and Hall: London, 1982; pp 505–534; (d) Porter, L. J. In *The Flavonoids. Advances in Research since 1980*; Harborne, J. B., Ed.; Chapman and Hall: London, 1988; p 27; (e) Geiger, H.; Quinn, C. J. In *The Flavonoids. Advances in Research since 1980*; Chapman and Hall: London, 1988.
2. Geiger, H.; Quinn, C. In *The Flavonoids*; Harborne, J. B., Mabry, T. J. H., Eds.; Academic Press: New York, 1975; pp 692–742.
3. Romanczyk, L. J.; Basak, A.; Townsend, C. A.; US Patent No. WO 0063201, 2000.; Tückmantel, W.; Kozikowski, A. P.; Romanczyk, L. J. *J. Am. Chem. Soc.* **1999**, *121*, 12073.
4. Kawamoto, H.; Nakatsubo, F.; Murakami, K. *Mokuzai Gakkashi* **1991**, *37*, 741.
5. Steynberg, P. J.; Nel, R. J. J.; Ferreira, D. *Tetrahedron* **1998**, *54*, 8153.
6. Burger, J. F. W.; Styenberg, J. P.; Young, D. A.; Brandt, E. V. *J. Chem. Soc. Perkin Trans. 1* **1989**, 671; Styenberg, J. P.; Burger, J. F. W.; Young, D. A.; Brandt, E. V.; Steenkamp, J. A.; Ferreira, D. *J. Chem. Soc. Perkin Trans. 1* **1988**, 3323.
7. Kawamoto, H.; Nakatsubo, F.; Murakami, K. *Synth. Commun.* **1996**, *26*, 531.
8. Basak, A.; Mandal, S.; Bandyopadhyay, S. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 1083.
9. Basak, A.; Nag, A.; Panchal, S.; Bhattacharya, G. *Biotechnol. Lett.* **1993**, *15*, 19.