LATVIJAS UNIVERSITĀTE

FUNKCIONALIZĒTU 2,6-DI-*TREŠ*-BUTILFENOLA ATVASINĀJUMU SINTĒZE UN ĶĪMISKO ĪPAŠĪBU IZPĒTE

Promocijas darbs doktora grāda iegūšanai ķīmijas nozarē organiskās ķīmijas apakšnozarē

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Promocijas darbs izstrādāts Latvijas Organiskās sintēzes institūtā laika posmā no 1992. gada līdz 1995. gadam.

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Darba aizstāvēšana notiks Latvijas Universitātes ķīmijas nozares habilitācijas un promocijas padomes [redacted] sēdē 1998. gada 19. martā plkst.$14^{\underline{00}}$ Latvijas Universitātes Ķīmijas fakultātē Kr. Valdemāra ielā 48.

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Padomes priekšsēdis:

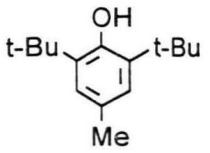
Satura rādītājs

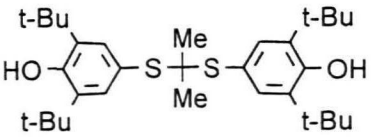
1. Ievads

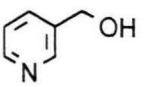
Ateroskleroze ir viena no mūsdienu visvairāk izplatītajām saslimšanām, kas it īpaši izplatīta industriāli attīstītajās valstīs. Pastāv daudz viedokļu par tās izcelsmes iemesliem un iespējām tos novērst. Nesen Ross[1] izvirzīja tā saukto "*response to injury*" teoriju par aterosklerozes ģenēzi. Saskaņā ar to savienojumi, kuri rada asinsvadu sieniņu endotēlija bojājumus, ir oksidatīvi modificēti zema blīvuma lipoproteīni (ox-LDL)[2]. Šos savienojumus ģenerē pats endotēlijs, un tie var izsaukt leikocītu migrāciju asinsvadu sieniņās, samazinot to elastību un izturību. No otras puses, ox-LDL izdalīšanās ietekmē augšanas faktoru sintēzi, tādejādi izraisot gludās muskulatūras šūnu proliferāciju, kas rada asinsvadu sieniņu sašaurinājumu[3].

Līdz ar to jau ilgāku laiku medicīnas ķīmijas jomā strādājošo zinātnieku uzmanību piesaista antioksidantu un lipīdu līmeni pazeminošo fragmentu kombinācija vienā molekulā. Tas ļautu pazemināt vispārējo LDL līmeni asinīs, tajā pašā laikā kavējot to oksidēšanos par ox-LDL.

Viens no plaši pazīstamiem antioksidantiem ir 2,6-di-*treš*-butil-4-metilfenols (**1**, butylated hydroxytoluene, BHT).





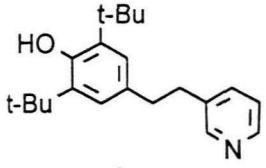


1, 2,6-di-*treš*-butil-4-metilfenols **2**, probukols **3**

Šī savienojuma spēja viegli veidot stabilu fenoksilradikāli ir labi pazīstama, un to izmanto kā tehnisko produktu vai pārtikas stabilizatoru. Analoģisks savienojums - 4,4'-(izopropilidēnditio)-bis-(2,6-di-*treš*-butilfenols) (**2**, probukols) ir labi pazīstams aģents holesterola līmeņa pazemināšanai asinīs[4].

Labi pazīstams hipolipidēmisks aģents ir nikotīnskābe vai, vēl labāk, savienojumi, no kuriem tā veidojas metaboliskā ceļā. Viens no populārākajiem šajā grupā ir piridil-3-karbinols (**3**)[5]:

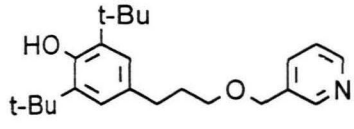
Jau 1989. gadā firmā Boehringer Ingelheim[6] sintezēja savienojumu **4**, kas apvieno sevī abus minētos farmakoforos fragmentus:



4

Šim preparātam atrasta augsta inhibējoša aktivitāte attiecībā pret vairākiem oksidējošiem enzīmiem, bet tā hipolipidēmiskā iedarbība, diemžēl, nav pētīta.

1993. gadā Golds un līdzstrādnieki[7] patentēja jauna tipa preparātu Nikanartīnu (**5**) ar augstu hipolipidēmisku aktivitāti, kam piemīt arī LDL oksidēšanos inhibējošas un gludās muskulatūras šūnu proliferāciju aizkavējošas īpašības[8,9]:



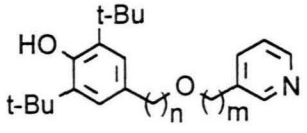
5, Nikanartīns

Līdz ar augstāk minētā tipa savienojuma parādīšanos pētījumu redzeslokā izvirzījās arī vairākas problēmas, kuru risināšana bija šī promocijas darba uzdevums:

1. BHT atvasinājumu antioksidantu īpašības balstās uz to spēju viegli veidot fenoksilradikāļus, kuri disproporcionējas, veido dimērus un aduktus ar tos inicējušajiem radikāļiem vai vidē esošajiem nukleofilus centrus saturošiem savienojumiem. Lai izvairītos no šādas BHT transformācijas, sarežģītāku savienojumu sintēzē izmanto aizsarggrupas. Arī aprakstītā Nikanartīna sintēze[7] ir sarežģīts 6 stadiju process, kura kopīgais iznākums ir aptuveni 25%. Tāpēc nepieciešams izpētīt, kādos reakciju apstākļos bez aizsarggrupu lietošanas iespējama minēto BHT atvasinājumu

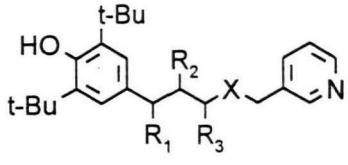
sintēze bez oksidācijas piemaisījumiem. Šai shēmai jādod praktisku iespēju iegūt lielākus preparāta daudzumus bioloģiskiem pētījumiem un jābūt piemērojamai rūpnieciskai sintēzei.

2. Tā kā BHT un nikotīnskābes prekursoru ēteri ir jauna savienojumu klase hipolipidēmisko antioksidantu grupā, trūkst informācijas par struktūras - aktivitātes likumsakarībām līdzīgu savienojumu rindā. Līdz ar to izvirzās uzdevums izstrādāt sintēzes metodes un iegūt sekojošu struktūru Nikanartīna analogus:



Kur n=1, 3;
m= 1, 3, 6

6, Nikanartīna analogi ar dažādu ķēdes garumu



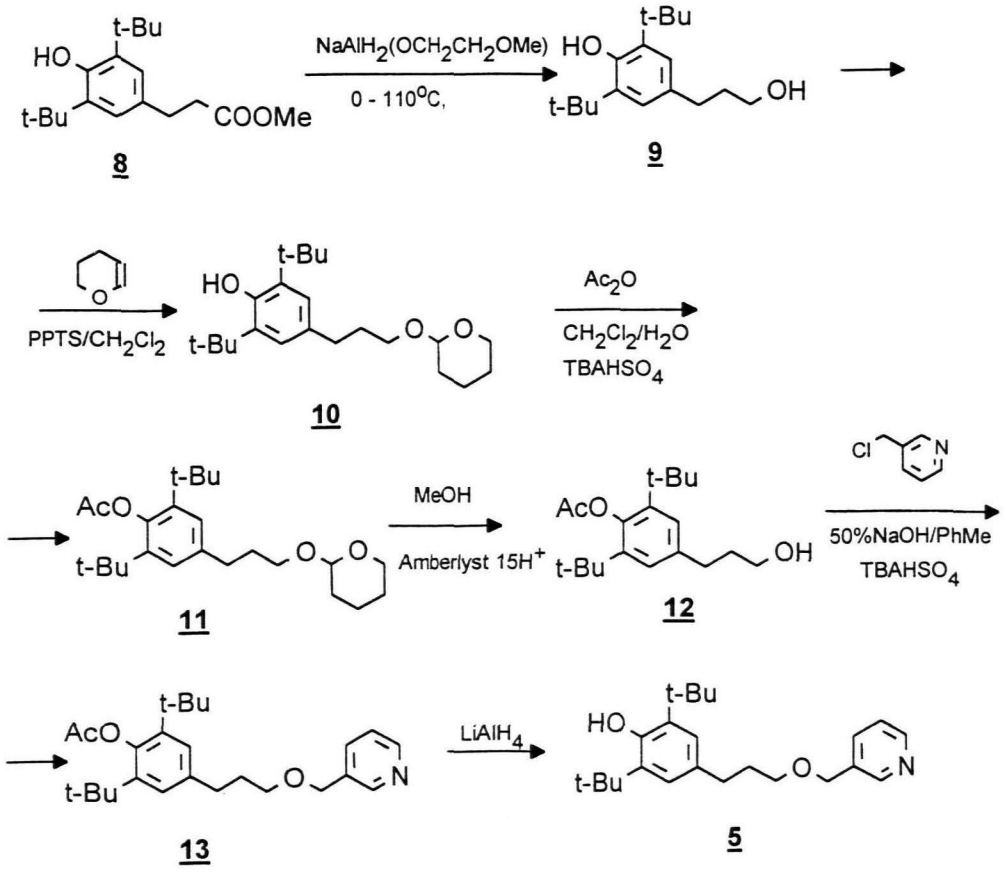
Kur R_1, R_2,R_3 - alkilaizvietotāji C_1-C_4
X - O vai S

7, Nikanartīna analogi ar sazarotu ķēdi.

3. Literatūrā nav ziņu par struktūras **6** un **7** savienojumu metabolismu. Tas nozīmē, ka jāveic selektīvu oksidēšanas reakciju pētījumi, kas palīdzētu noskaidrot Nikanartīna oksidatīvās biotransformācijas iespējamos virzienus un pagatavot potenciālo metabolītu standartus farmakoloģiskiem pētījumiem.

2. Nikanartīna sintēze

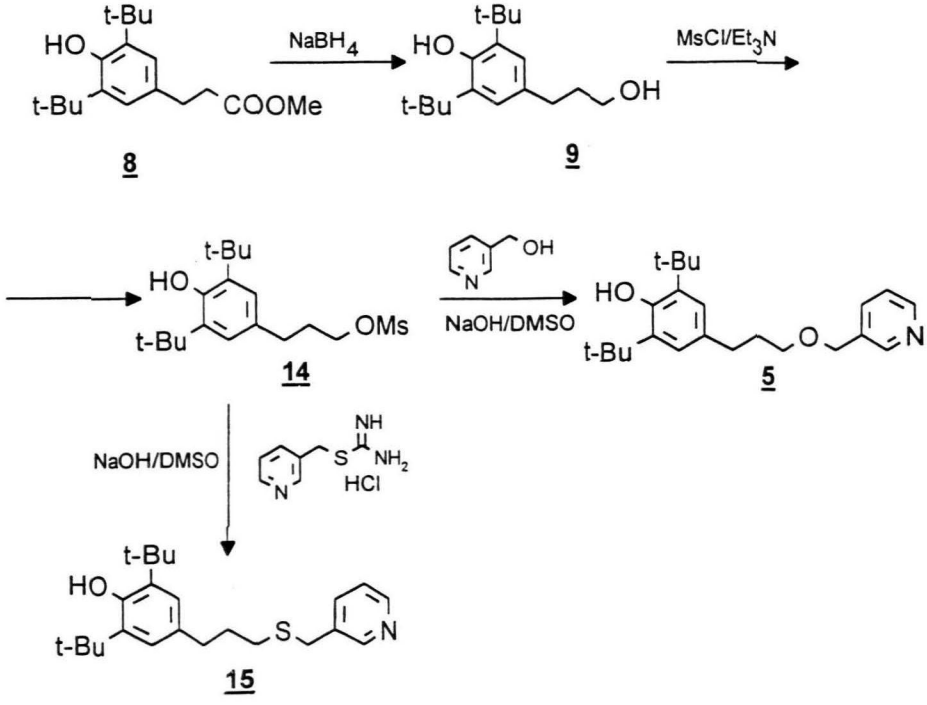
Literatūrā piedāvātais[7] Nikanartīna sintēzes ceļš atspoguļots sekojošā shēmā:



Galvenie trūkumi šajā shēmā ir lielais stadiju skaits un lielāku vielas daudzumu uzstrādei maz piemērotie reaģenti, kā, piemēram, litija alumohidrīds, nātrija bis-metoksietoksialumohidrīds, pikolilhlorīds. Jāņem vērā arī niecīgais kopējais iznākums visam procesam - 25% un ilgais laiks, kas nepieciešams tā realizācijai.

Ņemot vērā to, ka 2,6-di-*treš*-butilfenolu O-alkilēšanas reakcijas[10] notiek tikai stipru bāzu klātienē, mēs nolēmām izmēģināt shēmu bez aizsarggrupu ievešanas fenoliskajai hidroksilgrupai savienojumā **9**. Lai vēl vairāk samazinātu blakusreakciju iespēju šajā stadijā un vienlaicīgi izvairītos no nepieciešamības sintezēt pikolilhlorīdu, tika nolemts kā alkilētāju reaģentu izmantot nevis pikolilspirta atvasinājumu, bet no spirta **9** iegūstamu aģentu. Literatūrā ir aprakstīta di-*treš*-butilfenilpropanola **9** pārvēršana par atbilstošo bromīdu[11], bet tā ir iespējama tikai ļoti maigos apstākļos ar

oglekļa tetrabromīdu trifenilfosfīna klātbūtnē. Pārējās metodes, saskaņā ar literatūras datiem[12], dod sarežģītus produktu maisījumus. Tāpēc izvēlējāmies variantu, kurā spirtu vispirms pārvērš atbilstošajā mezilātā un tad izmanto kā alkilētāju. Patentā[7] piedāvātos fāzu pārneses reakcijas apstākļus nolēmām aizstāt ar alkilēšanu dimetilsulfoksīdā, kas izrādās ļoti piemērota Viljamsona ēteru sintēzes procesam[13]. Savukārt, reducējot esteri **8** par spirtu **9**, nātrija bis-metoksietoksialumohidrīdu plānojām aizvietot ar nātrija borhidrīdu, kurš reducē esterus, ja to aktivē ar metanolu t-butanolā[14]. Rezultātā tika izplānota un realizēta 3 stadiju sintēze, kura parādīta tālākajā shēmā:



8 **9** **14** **5** **15**

Pirmajā stadijā komerciāli pieejamo esteri **8** un nātrija borhidrīdu šķīdina t-butanolā un, lēni pievienojot metanolu, reducē par spirtu **9**. Šīs stadijas iznākums ir ap 90%, iegūtā produkta tīrība bez papildus apstrādes >95% saskaņā ar gāzhromatogrāfijas datiem, un to var viegli realizēt laboratorijas apstākļos ar 500g izejvielas iekrāvumu. 3,5-di-*treš*-Butilfenilpropanolu **9** benzola šķīdumā trietilamīna klātbūtnē apstrādā ar nelielu (≈5%) mezilhlorīda pārākumu un, ietvaicējot filtrātu, ar

gandrīz kvantitatīvu iznākumu iegūst mezilātu **14**, kurš pēc vienas kristalizācijas no izopropilspirta ir izmantojams pēdējā stadijā.

Procesa noslēdzošā un vienlaicīgi arī atslēgas stadija ir Viljamsona ēteru sintēze no mezilāta **14** un piridilkarbinola. Vispirms paaugstinātā temperatūrā dimetilsulfoksīda šķīdumā pikolilspirtu apstrādā ar smalki saberztu nātrija sārmu, un, pēc atdzesēšanas līdz 5-10°C, iegūtajam maisījumam pievieno mezilāta **14** šķīdumu dimetilsulfoksīdā. Stadijas iznākums ir tuvs kvantitatīvam, pēc vienas pārkristalizācijas no izopropilspirta gala produktā ar hromatogrāfiskām metodēm nevar konstatēt mezilāta **14** klātbūtni. Kopējais procesa iznākums ir lielāks par 75%. Izmēģinātie kondensāciju apstākļi un atbilstošie iznākumi apkopoti 1. tabulā:

1. tabula. Viljamsona ēteru sintēzes iznākumi dažādos apstākļos.

Npk.	Kondensācijas temperatūra, °C	n_{NaOH}/n_{ROMs}	$n_{PyCH2OH}/n_{ROMs}$	Reakcijas laiks, h	Iznākums, %
1	27	4.0	1.5	12	84.5
2	110	1.1	1.1	4.5	34.0*
3	25	1.5	2.0	2	67.0**
4	30	1.7	1.5	4.5	73.6**

*- produkts **5** attīrīts hromatogrāfiski

** - jēlprodukta iznākums >90%, bet konstatējamas mezilāta **14** pēdas, uzdots iznākums pēc attīrīšanas.

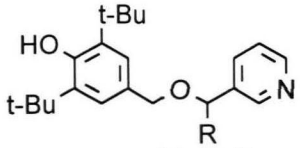
Izstrādātā metode, kā redzams iepriekšējā shēmā, izrādījās arī pietiekami universāla Nikanartīna tioanaloga **15** sintēzei. Pēc literatūrā aprakstītas metodes[15] ērti pagatavojams pikoliltiuronija hlorīds, kuru apstrādājot ar sārmu dimetilsulfoksīdā *in situ* rodas pikolilmerkaptāns. Tam, savukārt, reaģējot ar mezilātu **14** var iegūt nepieciešamo tioēteri **15**. Iznākums šajā reakcijā ir nedaudz vairāk kā 70%.

3. Nikanartīna analogu sintēze

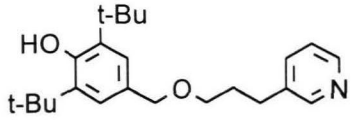
Šo uzdevumu nosacīti var sadalīt divās daļās. Viena daļa attiecas uz Nikanartīna analogu sintēzi ar dažādu ķēdes garumu starp BHT un piridilkarbinola grupām (skat. struktūru **6**), bet otra - alkilaizvietotāju ievadīšanu Nikanartīna molekulā, iegūstot preparāta analogus ar virknes sazarojumu starp abiem fragmentiem (**7**).

3.1. Nikanartīna analogu sintēze ar dažādu ķēdes garumu

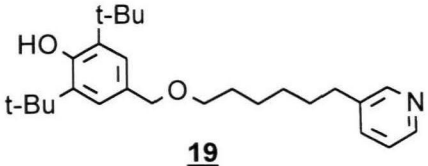
Šajā savienojumu grupā īpašu interesi izraisa vielas, kuru ētera saite no BHT grupas atdalīta ar vienu metilēngrupu:



16: R=H
17: R=Me



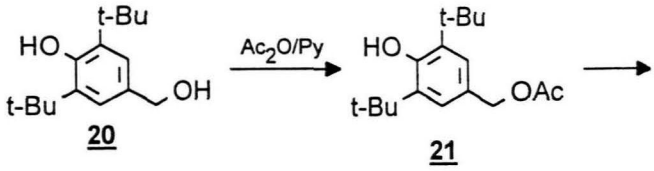
18

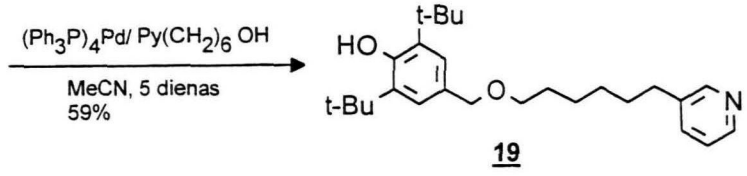


19

Ēteri **16** un **17** pēc C-O-C saites šķelšanas atbrīvo 3,5-di-*treš*-butil-4-hidroksifenilmetanolu (**20**), bet savienojums **17** nevar dot nikotīnskābi. Tādejādi bioloģiskajos testos vajadzētu novērot hipolipidēmiskās aktivitātes zudumu, kas apstiprinātu pieņēmumu, ka par šo iedarbību ir atbildīga tieši piridilkarbinola daļa. Savukārt ēteri **18** un **19** metaboliskajās pārvērtībās varētu dod nikotīnskābi, bet tikai pēc alkilķēdes oksidatīvās degradācijas[5]. Tam vajadzētu nodrošināt preparāta darbību ilgākā laika posmā.

Patentā[7] ir aprakstīta ētera **19** sintēze:



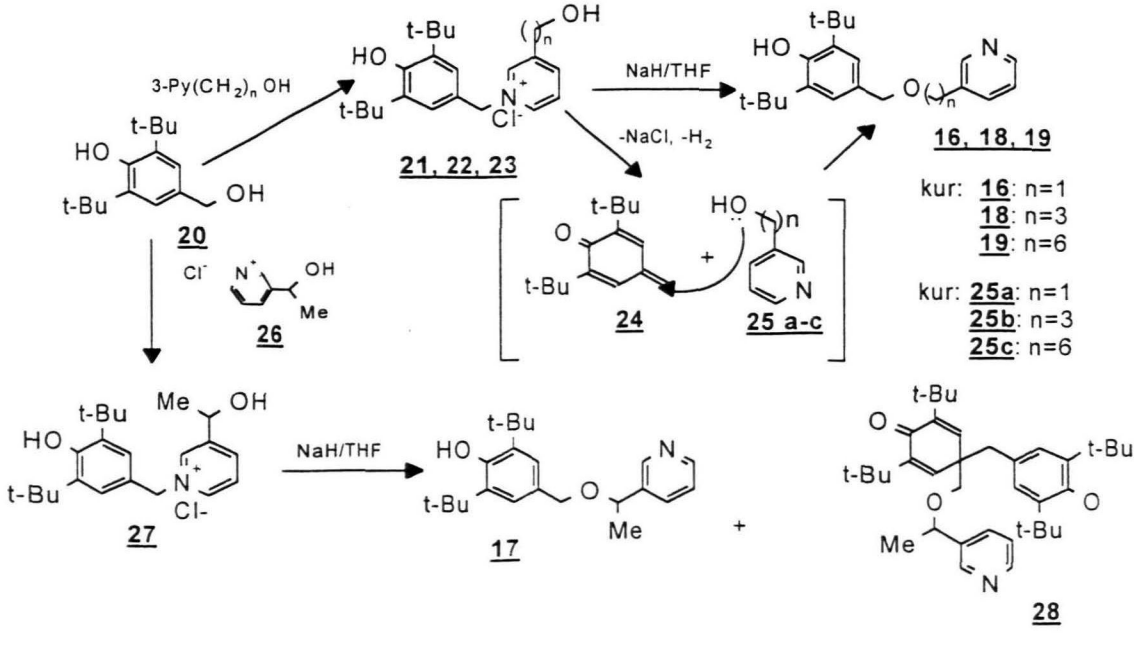


Tomēr reakcijas iznākums ir mazs, neskatoties uz izmantoto efektīvo katalizatoru.

Literatūrā[16] ir daudz norādījumu par ēteru sintēzi no 3,5-di-*treš*-butil-4-hidroksifenilmetanola (**20**), bet pamatā tie attiecas uz dažāda garuma alkilēteru iegūšanu un ietver abu komponentu vārīšanu skāba katalizatora klātbūtnē.

Tikai nedaudzas no mūsdienu metodēm piedāvā ēterifikācijas reakcijās izmantot spirtam **20** atbilstošos halogenīdus[17]. Atsevišķos gadījumos ieteikts izmantot N,N-dimetil-N-(3,5-di-*treš*-butil-4-hidroksibenzil)amīnu kā alkilētāju skābā vidē[18]. Jaunākos darbos iesaka ceturtējo acilamonija sāļu izmantošanu BHT grupējuma ievešanai molekulā[19]. Literatūrā minētas vairākas metodes, kuras paredz sākotnēju hinometīda **24** ģenerēšanu no 2,6-di-*treš*-butil-4-metilfenola **1**[20], vai benzilspirta **20** un sekojošas reakcijas ar nukleofiliem, tajā skaitā spirtiem, veidojot ēterus. Pārbaudot šo paņēmienu, piridilkarbinola **3** gadījumā izrādījās, ka metode var būt efektīva tikai izmantojot lielu spirta pārākumu, kas ir pieņemami reakcijās ar zemākajiem alkilspirtiem, bet pilnībā nepiemēroti izmantojot augstākos spirtus, kuri, pirmkārt, lēnāk reaģē ar hinometīdu, radot iespēju pēdējam dimerizēties[21], un, otrkārt, to pārākums grūti izvadāms no reakcijas vides produktu attīrot.

Izmantojot dažāda garuma piridilalkanolu ēteru sintēzei reakcijas variantu, kurā spirtam **20** jādarbojas kā alkilētājam, reakcijas maisījums ātri kļuva intensīvi krāsots pat strikti ievērojot visus priekšnoteikumus, kas izslēdz skābekļa piekļūšanu videi. Tomēr gaidītos ēterus reakcijas vidē neizdevās konstatēt pat zīmju līmenī. Brīvu piridilalkanolu vietā izmantojot atbilstošos hidrohlorīdus, jau pēc neilgas vārīšanas acetonitrilā parādījās nogulsnes, tomēr izrādījās, ka tie nav gaidītie ēteru hidrohlorīdi, bet gan piridīnija sāļi **21-23** un **27**. Visi turpmākie eksperimenti pierādīja, ka skābes klātbūtnē nav iespējams iegūt BHT un piridilkarbinolu ēterus. Lai noskaidrotu, vai pastāv iespēja realizēt piridīnija sāļu **21-27** izomerizāciju par ēteriem **16,18,19**, izmantojām Lādenburga N-alkilpiridīnija sāļu termiskās pārgrupēšanas pamatprincipu. Migrējošā alkilgrupa stājas pie piridīna molekulas elektronegatīvākā atoma - parasti C2 vai C4 oglekļa atoma. Mūs interesējošo piridīnu **21-23** molekulās elektronegatīvais centrs ir spirta grupas skābekļa atoms. Apstrādājot šo sāļu suspensiju tetrahidrofurānā ar nātrija hidrīdu, patiešām notiek pārgrupēšanās, kuras produkti ir ēteri **16-19**:



Pārgrupēšanās mehānisms vēl nav skaidrs, taču mēs pieņemam, ka, atraujot hlorīda anjonu piridīnija sālim **21-23**, molekula sabrūk, veidojot ļoti reaktīvu daļiņu - hinometīdu **24**, kurš reaģē ar vidē esošo nukleofilu - spirtu **25** un veido vajadzīgo ēteri. Eksperimentā ar otrējo spirtu **26** izdevās iegūt ēteri **17**, taču kā pamatproduktu izdalījām cikloheksadienona atvasinājumu **28**, kura rašanos var izskaidrot tikai ar hinometīda **24** klātbūtni reakcijas vidē. Bez tam, neskatoties uz pazemināto temperatūru un inertu atmosfēru, reakcijas maisījums satur daudz krāsainu produktu, kuru rašanos var izskaidrot ar hinometīda **24** uzkrāšanos reakcijas vidē[22]. Acīmredzot otrējais spirts ir stēriski traucēts, un pievienošanās reakcija norisinās lēnāk. Tādējādi reakcijas vidē uzkrājas hinometīds **24**, kas var dimerizēties, dodot raksturīgos krāsainos blakusproduktus.

Pirmējo spirtu ēteru iznākumi nav ievērojami atkarīgi no ķēdes garuma (iznākumu samazināšanās pārejot no **16** pie **18** un **19** drīzāk izskaidrojama ar zudumiem kristalizācijas laikā, jo 2. tabulā doti tīru vielu iznākumi).

2. tabula. Ēteru **16-23** iznākumi pārgrupēšanās reakcijā

Savienojums	Iznākums, %
16	94
17	24*
18	67
19	72

* ar 61% iznākumu izdalīts cikloheksadienona atvasinājums **28**.

Optimizējot reakcijas apstākļus savienojuma **16** sintēzei, mēģinājām izmantot citas bāzes hlorīdjona atraušanai. Iegūtie rezultāti atspoguļoti 3. tabulā.

Visos gadījumos, izņemot nātrija hidrīda reakciju, pat pazeminātā temperatūrā rodas ievērojams daudzums blakusproduktu. Neapšaubāmi, sausa nātrija hidrīda lietošana reakcijā nav pievilcīga no preparatīvā viedokļa, jo sākumā prasa zināmu

aktivēšanu sasildot, bet pēc reakcijas sākuma, uz ko norāda ūdeņraža izdalīšanās, atkal strauju atdzesēšanu, lai novērstu blakusproduktu rašanos.

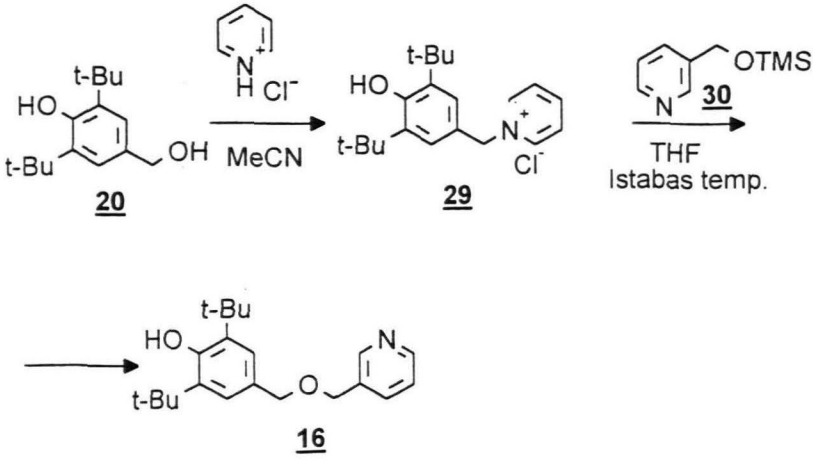
3. tabula. (3,5-di-*trešt*-Butil-4-hidroksifenil)metil-(3-piridil)metil ētera **16** sintēze

N.p.k.	Bāze / Šķīdinātājs	Laiks / T, (°C)	Iznākums (%)
1.	NaH / THF	3h / -15	94
2.	NaH / THF	2h / 20	45
3.	BaO / MeCN	3h / vārot	41
4.	K_2CO_3 / MeCN	5h / vārot	40
5.	K_2CO_3 / DMF	1h / vārot	zīmes[a)]
6.	Et_3SiH / THF	24h / 20	nav reakcijas.
7.	DABCO / THF	24h / 20	0[b)]
8.	NaOMe /MeCN	2h / vārot	zīmes[a)]

[a)]- ļoti daudz krāsainu reakcijas produktu, nelielu daudzumu (<1%) ētera **16** var izdalīt hromatogrāfiski.

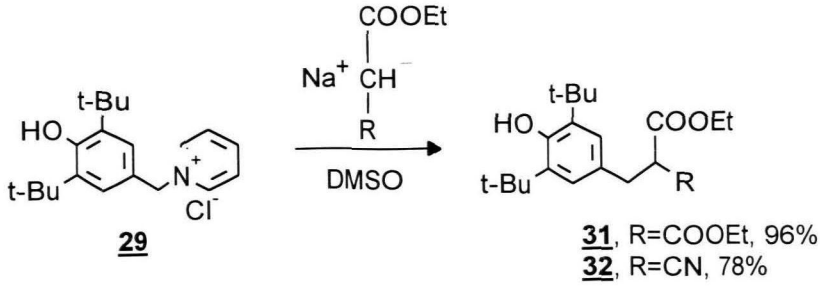
[b)]- reakcijas maisījumā var konstatēt brīvu piridilkarbinolu (GC) un alkilētu DABCO

Lai izvairītos no minētajām grūtībām, izmēģinājām sililēteru metodi[23]. Vispirms piridīna hidrohlorīda reakcijā ar 3,5-di-*treš*-butil-4-hidroksifenilmetanolu (**20**) praktiski ar kvantitatīvu iznākumu pagatavojām piridīnija sāli **29**. Apstrādājot piridīnija sāli **29** ar O-trimetilsililpiridilkarbinolu **30** tetrahidrofurānā, ieguvām vajadzīgo ēteri **16** ar 82% iznākumu:



Paralēli minētajam pielietojumam pētījām arī citas iespējas izmantot piridīnija sāli **29**. Literatūrā aprakstīta[24] metode 3,5-di-*treš*-butil-4-hidroksifenilmetilgrupas

ievadīšanai molekulā, izmantojot atbilstošo halogenīdu reakcijas. Šīs metodes pamattrūkumi ir nepietiekami augstais iznākums un arī *bis*-alkilēšanas produktu klātbūtne reakcijas maisījumā. Mēs piedāvājam savu reaģentu - piridīnija sāli **29** šādu alkilēšanas reakciju veikšanai. Pārbaudes eksperimenti ar malonesteri un ciānetiķesteri rāda, ka iespējams iegūt tīrus produktus ar augstu iznākumu:



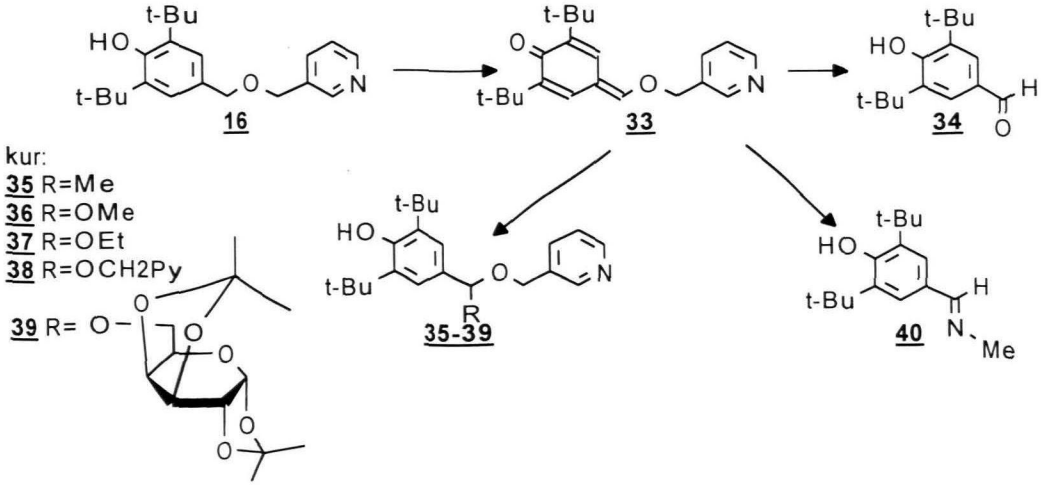
29

31, R=COOEt, 96%
32, R=CN, 78%

Optimāli reakcijas apstākļi - dimetilsulfoksīds istabas temperatūrā.

Kopumā pētījumu rezultātā, izmantojot stēriski traucētu fenolu klases īpatnības, radīta jauna metode 3,5-di-*treš*-butil-4-hidroksifenilmetilēteru sintēzei ar augstu iznākumu un tīrības pakāpi, kura ir vienkārša lietošanā. Paralēli izstrādāts jauns reaģents 3,5-di-*treš*-butil-4-hidroksifenilmetilgrupas ievadīšanai pie oglekļa atoma reakcijās ar karbanjoniem.

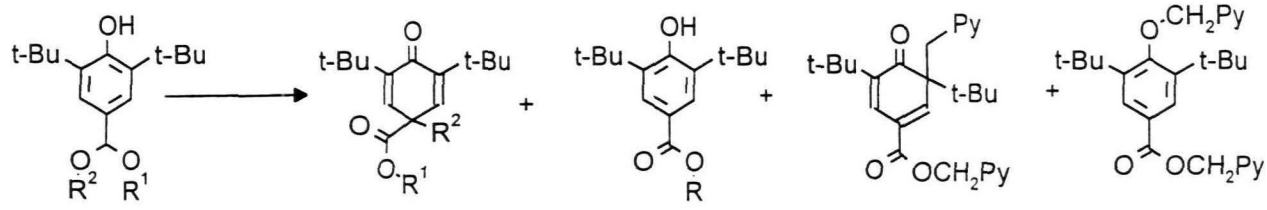
Turpinot ētera **16** ķīmisko īpašību pētīšanu, apskatījām iespējas ievadīt dažādus aizvietotājus benziliskajā metilēngrupā.



Oksidējot savienojumu **16** ar sarkano asinssāli bāziskā vidē, ieguvām relatīvi stabilu hinometīdu **33**. Savienojumu **33** var pārkristalizēt no heksāna, sausā veidā tas ir uzglabājams vairākas dienas, kamēr šķīdumā vai uz silikagela ātri parādās aldehīda **34** piemaisījums. Reakcijās ar nukleofiliem viegli rodas pievienošanās produkti **35-39**. Reakcijas produkts ar etilmagnija bromīdu **35** ir stabils, turpretī spirtu pievienošanās produkti **36-39** ir acetāla struktūras un šķīdumos sadalās mitruma iedarbībā, veidojot aldehīdu **34**. Izdarot eksperimentu dimetilsulfoksīdā ar 25% ūdens piedevu, pēc 8 dienu izturēšanas istabas temperatūrā konstatēts 30% aldehīda **34** piemaisījums acetālā **38**.

Atšķirībā no O-nukleofiliem reakcijā ar metilamīnu vispār neizdodas iegūt gaidīto aminālu, tā vietā rodas imīns **40**.

Negaidīts rezultāts tika iegūts, oksidējot acetālus **36, 38** un dimetilacetālu **41** ar sarkano asinssāli bāziskos apstākļos.



36 R_1=CH_2(3-Py); R_2=Me
38 R_1=R_2= CH_2(3-Py)
41 R_1=R_2=Me

42a R_2=CH_2(3-Py); R_1=Me
42b R_1=R_2= CH_2(3-Py)

43a, R=CH_2Py
43b, R=Me

44

45

Reakcijā rodas produktu **42-46** maisījums, kura sastāvs ir atkarīgs no acetāla struktūras un atspoguļots 4. tabulā.

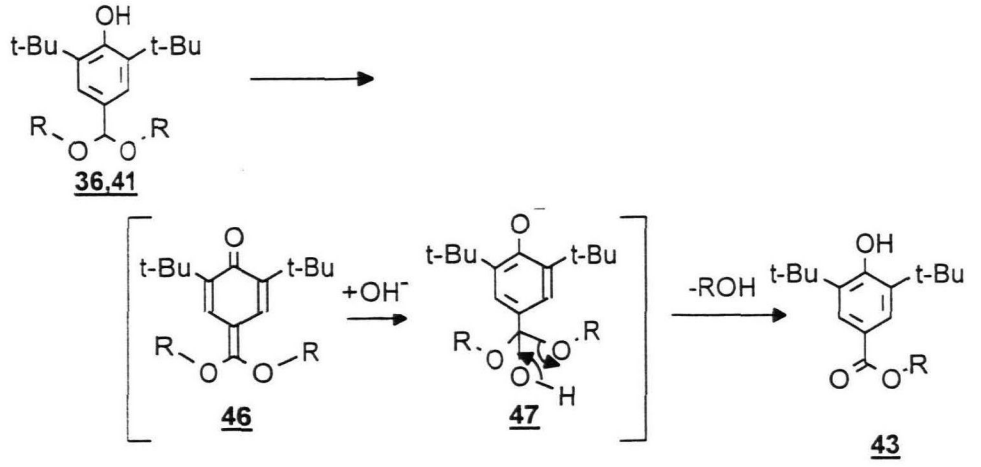
Gadījumā, kad R_1=R_2=(3-Py)CH_2, dominējošais produkts (76% izdalīta iznākuma) ir esteris **42b**, aromātiskais esteris **43a** rodas ar aptuveni 10% iznākumu, bet atvasinājumu **44** un **45** iznākumi ir 2-3% robežās. Aizvietojot vienu no pikolilgrupām

ar metilgrupu, ar 90% iznākumu tiek izdalīts *ipso*-pievienošanās produkts **42a**, kamēr dimetilacetāls **41** ar 91% iznākumu dod atvasinājumu **43b**.

4. tabula. 3,5-di-*treš*-Butil-4-hidroksibenzaldehīda acetālu oksidēšana

R^1	R^2	Iznākumi, (%)			
		42	**43**	**44**	**45**
CH_2(3-Py)	CH_2(3-Py)	76	10	2	3
Me	CH_2(3-Py)	90	-	-	-
Me	Me	-	91	-	-

Pieļaujot hinometīda **46** rašanos reakcijā, var izskaidrot estera **43** veidošanos caur ūdens pievienošanu oksidācijas produktam **46** un ortoestera **47** sabrukšanu, izšķeļot vienu molekulu spirta. Šī pēdējā stadija aprakstīta lieratūrā un realizējas, oksidējot dažadus acetālus[25,26].



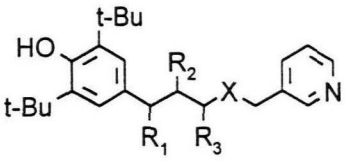
Savukārt *ipso*-pievienošanās produkti **42** un **44**, kā arī fenilēteris **45**, kurus izdala no reakcijas vides nav izskaidrojami minētās shēmas ietvaros. Šīs pārvērtības mehānisms patlaban ir nenoskaidrots, bet neapšaubāma ir fenoksilradikāļa rašanās pirmajā stadijā, uz ko norāda zilganzaļais krāsojums reakcijas sākumā, kam pamazām pazūdot, maisījumā vairs nevar konstatēt arī izejvielu.

Iegūtie rezultāti parāda, ka hinometīdu sistēmas ir ļoti ērts sintētisks instruments dažādu atvasinājumu pagatavošanai. Izmantojot hinometīdu reakcijas, iespējams

ievērojami vienkāršot garas sintētiskas shēmas ar dārgu katalizatoru un aizsargrupu pielietošanu.

3.2. Sazarotas virknes Nikanartīna analogu sintēze

Sazarotas 4-oksa- un 4-tiapentāna virknes konstruēšana Nikanartīna molekulā ļauj iegūt vairāk informācijas par struktūras - aktivitātes likumsakarībām šādu savienojumu rindās.



Kur R_1, R_2,R_3 - alkilaizvietotāji C_1-C_4

X - O vai S

7, Nikanartīna analogi ar sazarotu ķēdi.

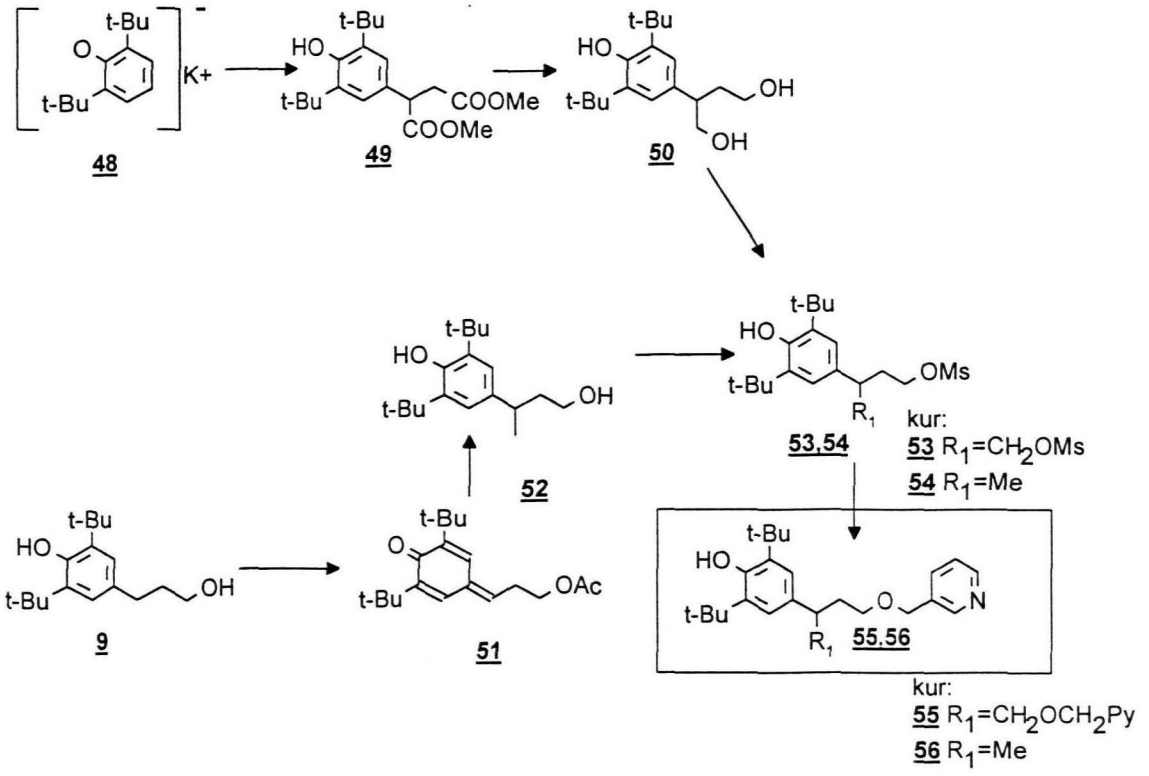
Aizvietotājs R_1 pie α–oglekļa atoma pret fenola gredzenu, visticamāk, varētu ietekmēt fenoksilradikāļa veidošanos, tādējādi izmainot visas molekulas redokspotenciālu un, līdz ar to, arī antioksidanta aktivitāti. Savukārt, ievadot molekulā aizvietotāju R_3, tiktu telpiski apgrūtināta pieeja ētera saitei un tās hidrolīze organismā. Tas izraisītu hipolipidēmiskās aktivitātes izmaiņu un varētu paildzināt preparāta darbību. Visbeidzot, aizvietotājs R_2 var izmainīt visas molekulas kopējo lipofilitāti, tādejādi ietekmējot preparāta uzsūkšanās spēju organismā.

Turpmāk tiks apskatīti katra atsevišķa aizvietotāja ievadīšanas paņēmieni molekulā.

3.2.1. Aizvietotājs α–stāvoklī pret fenola gredzenu

Izmantojot mūsu jau iepriekš izstrādāto taktiku, varētu secināt, ka galvenais priekšnoteikums alkilaizvietotāju ievadīšanai α–stāvoklī pret fenola gredzenu būtu pagatavot atbilstošos, propanolam **9** analogos, spirtus. No otras puses, iespējamas 4. stāvoklī neaizvietota 2,6-d-*treš*-butilfenola reakcijas ar alkilētājiem vai α,β–nepiesātinātiem karbonilsavienojumiem[27] tādā veidā, lai uzreiz iegūtu izejvielu vajadzīgā spirta pagatavošanai.

Mēs kombinējām šīs divas pieejas un realizējām sekojošu shēmu:



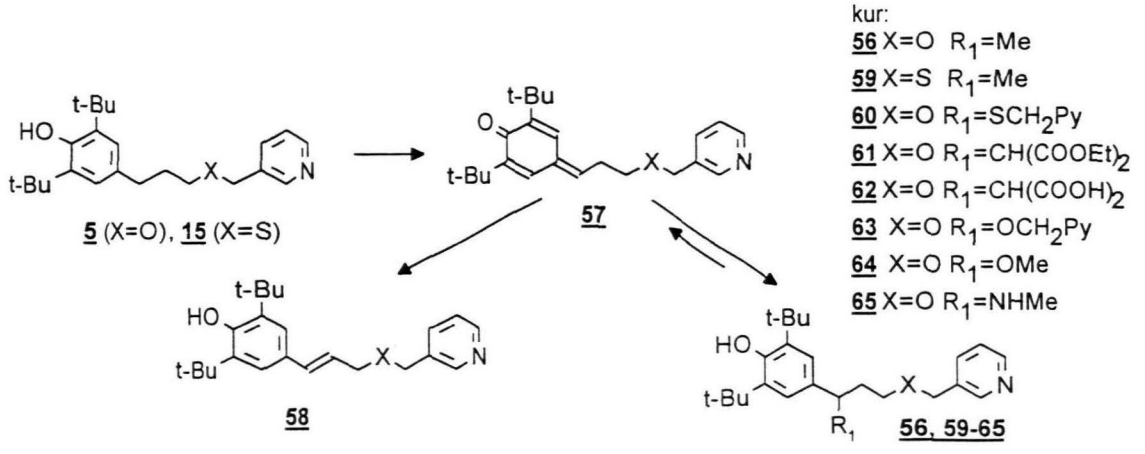
Apstrādājot fenola kālija sāli **48** ar dimetilmaleātu pēc literatūrā aprakstītām metodēm var iegūt *bis*-esteri **49**, kura reducēšana ar litija alumohidrīdu ar labu iznākumu dod butāndiolu **50**. Tālākajā reakcijā ar mezilhlorīdu var pagatvot *bis*-mezilātu **53**. Diemžēl fenolāta **48** reakcija ar metilkrotonātu notiek ļoti lēni pat 150°C temperatūrā aizkausētā ampulā. Līdz ar to šī metode izrādījās pilnīgi neizmantojama α–metilatvasinājumu sintēzei.

Alkilradikāļu ievadīšanai α-stāvoklī pret fenola gredzenu mēs izstrādājām savu sintēzes taktiku, kura balstās uz vairākām stēriski traucēto fenolu īpatnībām.

Vispirms, apstrādājot propilspirtu **9** ar acetanhidrīdu piridīnā, var ar labu iznākumu selektīvi acilēt alifātisko hidroksilgrupu. *treš*-Butilgrupu stēriskā ietekme pilnībā novērš fenola acetilēšanu un padara nevajadzīgu patentā[7] aprakstīto aizsargrupas izmantošanu. Iegūto acetātu oksidē ar sarkano asinssāli bāziskā vidē[28],

ģenerējot hinometīdu. Šajā gadījumā iegūtais hinometīds **51** ir stabils savienojums, kuru, kristalizējot no heksāna, var ērti attīrīt. Produkta iznākums, rēķinot uz spirtu **9** ir aptuveni 60%. Ņemot vērā literatūras datus par dažādu hinometīdu reakcijām ar nukleofiliem[29], apstrādājām produktu **51** ar metilmagnija jodīda pārākumu ēterī. Tādejādi ar vairāk nekā 95% iznākumu var iegūt nepieciešamo spirtu **52**. Šī metode ir pietiekami universāla jebkuru alkilaizvietotāju ievadīšanai α-stāvoklī pret fenola gredzenu 3-(3,5-di-*treš*-butil-4-hidroksifenil)propanolā **9**.

Tālāk turpinot pētījumus par hinometīdu sistēmu īpašībām, mēs mēģinājām oksidēt Nikanartīnu. Parastā apstrāde ar kālija ferricianīdu baziskā vidē deva spilgti dzeltenu eļļu **57**. Šis savienojums istabas temperatūrā ir nestabils un aptuveni divu dienu laikā izomerizējas par daudz stabilāko stirolu **58**. Izomerizāciju katalizē gan bāzu, gan skābju klātbūtne[30]. Savienojumu **57** var attīrīt, hromatografējot uz *Silasorb 600*, kamēr apstrāde ar *Kieselgel 60* pilnībā izomerizē hinometīdu par stirolu **58**.



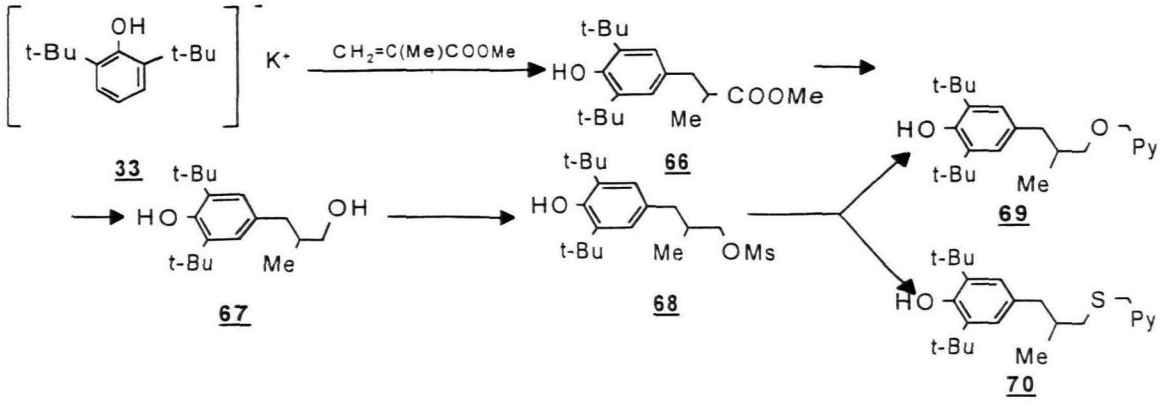
Hinometīda **57** reakcijās ar dažādiem nukleofiliem viegli rodas pievienošanās produkti **59-65**. Tomēr jāatzīmē, ka paralēli vēlamajiem produktiem gandrīz vienmēr var izdalīt arī izomerizācijas produktu - stirolu **58**. Reakcijās ar oglekļa un sēra nukleofiliem iegūtie produkti (**56, 59-62**) ir stabili, kamēr mijiedarbība ar skābekļa un slāpekļa nukleofiliem ir apgriezeniska, un pat no tīriem produktiem (**63-65**) vairāku

secīgu kristalizāciju rezultātā var iegūt stirolu **58**, kurš acīmredzot ir termodinamiski stabilāks par hinometīdu **57**. Jāatzīmē, ka atšķirībā no iepriekš minētā hinometīda **33**, tā analogs **57** reakcijā ar metilamīnu veido stabilu aduktu **65**. Savukārt iepriekš aprakstītie acetāli **36,38** un **41** ir stabilāki par šajā nodaļā apskatītajiem alkoksiatvasinājumiem. Tomēr, realizējot nukleofīlu pievienošanos hinometīdam **57** kinētiskās kontroles apstākļos, var vienā stadijā ar labiem (>70%) iznākumiem iegūt dažādus Nikanartīna atvasinājumus, kurus būtu grūti pagatavot, izmantojot klasiskās metodes.

3.2.2. Aizvietotājs β–stāvoklī pret fenola gredzenu

Diemžēl, hinometīdu reakcijas nevar izmantot aizvietotāju ievadīšanai β–stāvoklī pret fenola gredzenu. Līdz ar to, lai pagatavotu šādus Nikanartīna atvasinājumus, jāatgriežas pie sākotnēji izstrādātās shēmas. Atslēgas stadija šīm sintēzēm ir 3-(3,5-di-treš-butil-4-hidroksifenil)propanola **9** atbilstošo atvasinājumu iegūšana, kurus Viljamsona ēteru kondensācijas apstākļos varētu pārvērst par vajadzīgo pikolilēteri vai tā tioanalogu.

Aprakstīta[31] savienojuma **67** sintēzes metode, pievienojot 2,6-di-treš-butilfenola kālija sāli **33** α,β-nepiesātināto karbonskābju esteriem un reducējot iegūto produktu **66** līdz spirtam **67**.



Tomēr šāda paņēmiena pielietojamību ierobežo attiecīgo akrilātu pieejamība, jo vienīgais rūpnieciskais reaģents ir metilmetakrilāts.

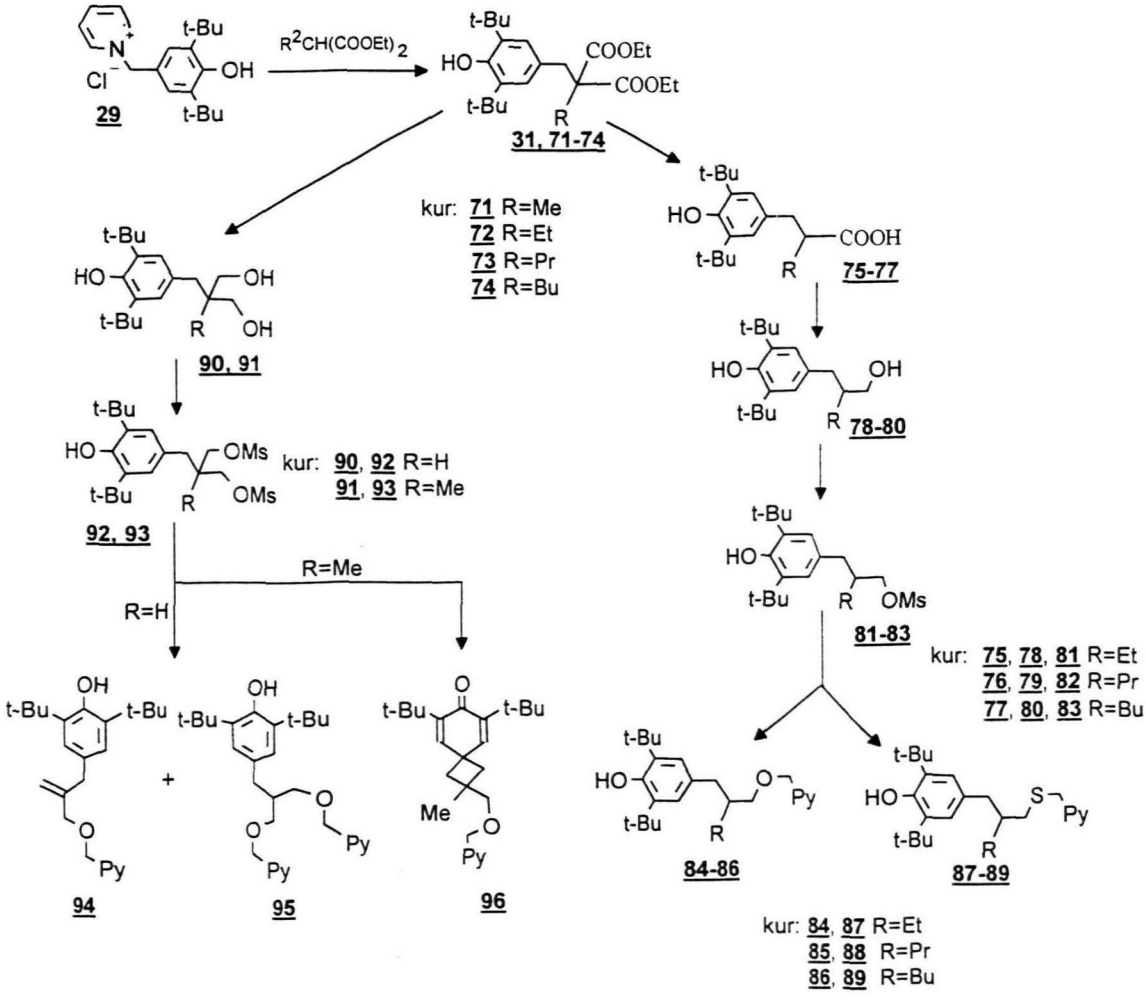
Mūsu piedāvātajā shēmā izejviela β–alkilētiem 3-(3,5-di-treš-butil-4-hidroksifenil)-propanoliem ir atbilstošie malonesteri, kurus var pagatavot no dietilmalonāta. Izmantojot iepriekš aprakstīto benzilēšanas reaģentu - piridīnija sāli **29,** var viegli iegūt diesterus **31** un **71-74**. Iznākumi šajās stadijās pārsniedz 80%. Diesterus **71-74** hidrolizē bāziskā vidē, un iegūtās dikarbonskābes dekarboksilē vakuumā, izdalot karbonskābes **75-77**, kuras reducējot dod spirtus **78-80** ar alkilaizvietotāju vajadzīgajā pozīcijā.

Izmantojot mūsu izstrādāto ēteru sintēzes metodi, propanolus **78-80** pārvērš atbilstošajos mezilātos **81-83**. No tiem Viljamsona reakcijas apstākļos var pagatavot ēterus **84-86** vai tioēterus **87-89**. Kopējais produktu iznākums ir 30-55%, atkarībā no aizvietotāja lieluma.

Mezilātu **81-83** reakcijai ar piridilkarbinolu nepieciešama augstāka temperatūra nekā atbilstošajam neaizvietotajam alkilētājam **14**. Kvalitatīvai reakcijas produktu attīrīšanai nepieciešams izmantot hromatogrāfiju.

Nedaudz atšķirīgi reakcija notiek, mēģinot iegūt Nikanartīna analogu ar diviem piridilkarbinola atlikumiem molekulā. Šajā nolūkā, reducējot esterus **31** un **71**, tika pagatavoti dioli **90** un **91,** kuru pārvēršana par atbilstošajiem mezilātiem **92** un **93** norisinās viegli, taču ēteru sintēzes apstākļos (reakcija abos gadījumos sākas tikai virs 80°C) novērojamas principiāli atšķirīgas reakcijas atkarībā no aizvietotāja R. Ja R=H, līdzās gaidītajam bis-ēterim **95** rodas ievērojams daudzums eliminēšanās produkta **94**. Gadījumā, ja molekula satur vēl vienu metilgrupu pie β-oglekļa (R=CH_3), *bis*-ēteris nerodas vispār, bet ar labu iznākumu izdalīts spirocikls **96**. Literatūrā[32] apskatīta 2,6-

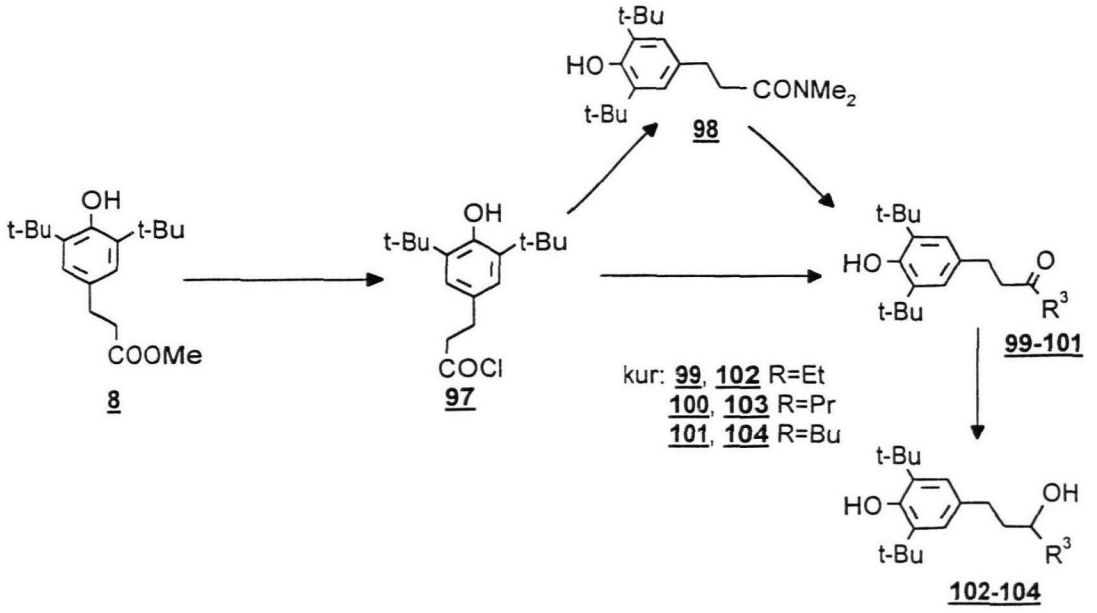
di-*treš*-butilfenola spirociklisko ciklopropil- un ciklopentilatvasinājumu sintēze, taču ciklobutānu saturošu spirociklisku sistēmu rašanās šādās reakcijās līdz šim nav aprakstīta. Neskatoties uz to, spirocikls **96** ir vienīgais reakcijā izdalītais produkts, ko izdodas izolēt ar 47% iznākumu. Vēl reakcijas maisījumā var konstatēt diola **91** klātbūtni.



3.2.3. Aizvietotājs γ–stāvoklī pret fenola gredzenu

Analoģiski iepriekšējā nodaļā aprakstītajiem savienojumiem arī γ-aizvietotu Nikanartīna atvasinājumu sintēzei nepieciešamās izejvielas ir atbilstošie spirti ar alkilradikāli vajadzīgajā pozīcijā.

Racionāli ir sākt sintēzi no komerciāli pieejamā estera **8**. Hidrolīze un tai sekojoša iegūtās karbonskābes reakcija ar tionilhlorīdu dod hlorīdu **97**.



Acilhlorīdu izmantošanai ketonu sintēzē ir vairākas vispārīgas metodes. Darba gaitā tika izmēģinātas un salīdzinātas divas no tām:

A) acilhlorīda pārvēršana par dimetilamīdu un sekojoša reakcija ar Grinjāra reaģentu[33];

B) acilhlorīda reakcija ar Grinjāra reaģentu Fe (III) acetilacetonāta klātbūtnē[34].

Abu metožu salīdzinājums dots 5. tabulā:

5. tabula. Ketonu **99-101** sintēze no 3-(3,5-di-*treš*-butil-4-hidroksifenil)propionil-hlorīda **97**

N.p.k.	Savienojuma Nr.	Aizvietotājs R	Metode	Reakcijas laiks,h	T, ^{O}C	Iznākums, %
1	99	Et	B	2.5	0	53
2	99	Et	A	48	vārot THF	34
3	100	Pr	B	2.5	0	95
4	100	Pr	A	48	vārot THF	43
5	101	Bu	B	-	-	-
6	101	Bu	A	170	ist. temp.	73

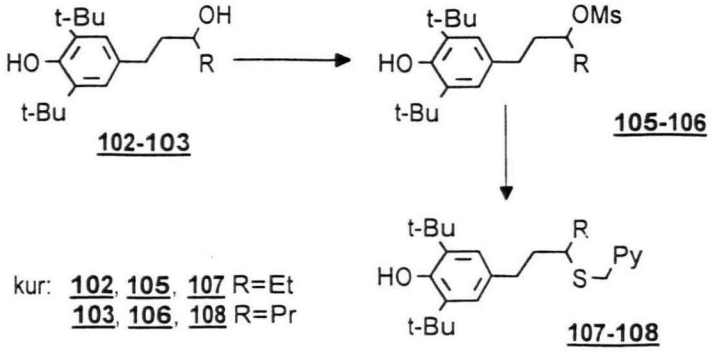
Kaut arī **B** metode kopumā dod labākus iznākumus un process notiek ātrāk, reakciju būtiski ietekmē hlorīda **97** kvalitāte. Pat ļoti neliels hlorūdeņraža piemaisījums izjauc reakcijas stehiometriju, patērējot Grinjāra reaģentu. Savukārt acilhlorīda **97** attīrīšana ir sarežģīts uzdevums, jo savienojums šķīdumos ir nestabils un polimerizējas.

Ketonus **99-101** ar litija alumohidrīdu pazeminātā temperatūrā var reducēt par atbilstošajiem spirtiem **102-104**. Reducēšanas iznākumi ir tuvi kvantitatīviem (sk.6. tabulu).

6. tabula. Spirtu **102-104** iegūšana

N.p.k.	Savienojuma Nr.	Aizvietotājs R	T, ^{O}C	Iznākums,%
1	102	Et	-50	98
2	103	Pr	-35	93
3	104	Bu	-45	98

No iegūtajiem spirtiem **102** un **103** pazeminātā temperatūrā var pagatavot arī atbilstošos mezilātus **105** un **106**, taču to reakcijā ar pikolilspirtu rodas tikai eliminēšanās produktu maisījums. Turpretī pikolilmerkaptāns, būdams stiprāks nukleofils, veido gaidītos tioēterus **107** un **108** ar 45-50% iznākumu.



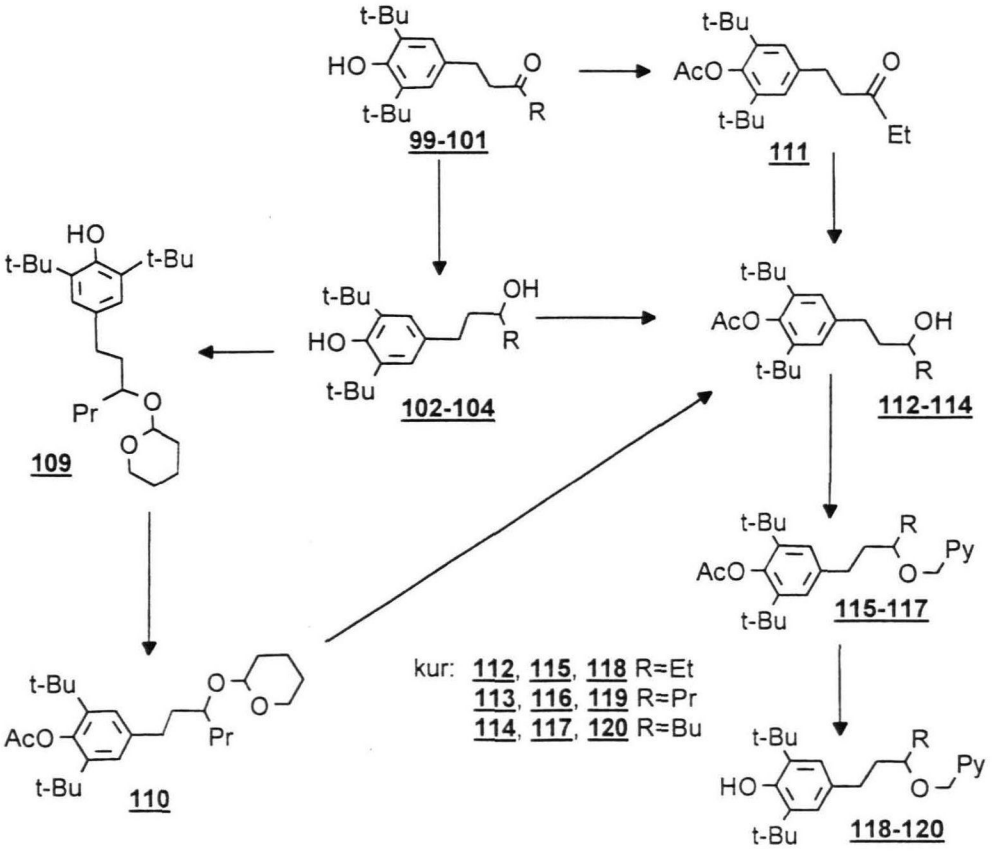
Lai iegūtu nepieciešamos ēterus, alkilēšanas reakcijā ar pikolilhlorīdu nepieciešams aizsargāt spirtu **102-104** fenoliskās hidroksilgrupas, tās acetilējot[7]. Literatūrā[7] aprakstītā procedūra, izmantojot THP aizsardzību (**109**), acetilēšanu (**110**) un sekojošu

aizsargrupas noņemšanu, deva 37% iznākumu gadījumā, ja R=Pr (**113**). Mums izdevās atrast apstākļus selektīvai fenoliskās hidroksilgrupas acetilēšanai, neizmantojot aizsargrupas.

7. tabula. Spirtu **102-104** tiešā acetilēšana

Npk.	Produkta Nr.	Aizvietotājs R	Laiks, h	Iznākums,%
1	112	Et	1	62
2	113	Pr	3	64
3	114	Bu	2	71

Tālākas metodes optimizācijas gaitā izstrādājām paņēmienu, pēc kura vispirms acetilē ketonu **99** un pēc tam maigos apstākļos ($LiAlH_4$, -50°C) selektīvi reducē acetāta **111** karbonilgrupu, iegūstot vajadzīgo spirtu **112**.



Ēteru kondensācija fāzu pārneses apstākļos[7] (**115-117**) un sekojoša acetilgrupas noņemšana ar litija alumohidrīdu tetrahidrofurānā dod vajadzīgos produktus (**118-120**).

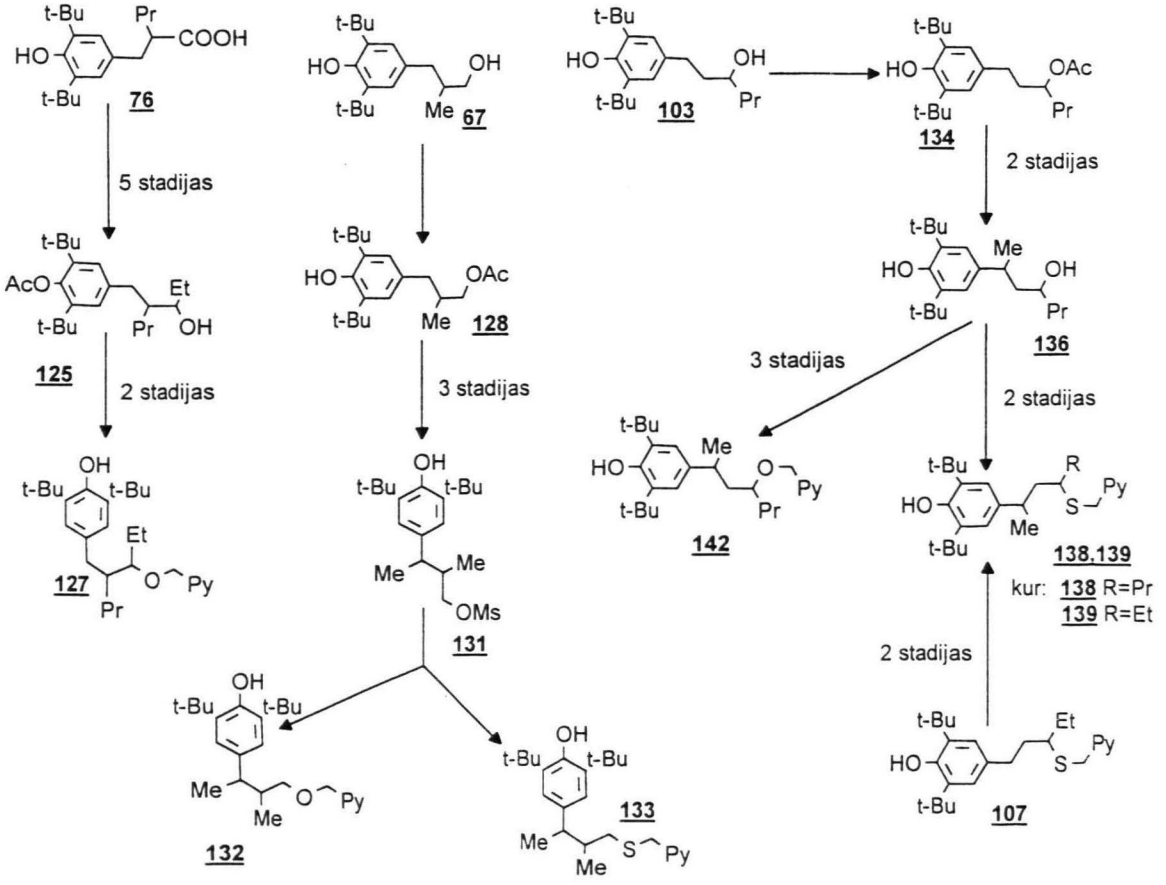
8. tabula. γ–Aizvietotu pikolilēteru **118-120** sintēze

N.p.k.	Produkta Nr.	Aizvietotājs R	Iznākums kondensācijai, %	Iznākums deacilēšanai, %	Kopīgais iznākums, %
1	118	Et	-	-	39
2	119	Pr	-	-	42
3	120	Bu	49	89	44

Kondensācijas un acetilgrupas noņemšanas stadiju atsevišķie iznākumi noteikti ne visos gadījumos, jo ilgāku laiku apstrādājot ēterus **115-117** ar sārma šķīdumu fāzu pārneses katalīzes apstākļos, acetilgrupa daļēji hidrolizējas.

3.2.4. Diaizvietotu Nikanartīna atvasinājumu sintēze

Izmantojot iepriekš aprakstītās metodes, pagatavoti arī vairāki diaizvietota Nikanartīna analogi.



Tā, no karbonskābes **76** 5 stadijās caur hlorīdu **121** un dimetilamīdu **122** var pagatavot ketonu **123,** kuru acetilējot par esteri **124** un reducējot to zemā temperatūrā,

iegūst nepieciešamo spirtu **125**. Eterificēšanas reakcija fāzu pārneses apstākļos (**126**) ar sekojošu O-acilgrupas noņemšanu dod β,γ-diaizvietota Nikanartīna analogu **127.**

Savukārt, no iepriekš aprakstītā 3-(3,5-di-*treš*-butil-4-hidroksifenil)propanola **67**, to apstrādājot ar acetanhidrīdu piridīnā, selektīvi ieguvām acetātu **128**. Pēdējo oksidējot ar sarkano asinssāli benzola un sārma ūdens šķīduma emulsijā, rodas stabils hinometīds **129**, kura reakcija ar metilmagnija jodīda pārākumu dod α,β–diaizvietotu spirtu **130**. Šādam pirmējam spirtam ir pielietojama iepriekš aprakstītā Viljamsona ēteru sintēze caur mezilātu **131**, kas reakcijā ar piridilkarbinolu vai atbilstošo tiolu dod ēteri **132** vai attiecīgi tioēteri **133**.

α,γ–Diaizvietotu Nikanartīna atvasinājumu sintēzē par izejvielu var izmantot γ–aizvietoto spirtu **103**. Tā apstrāde ar acetanhidrīdu piridīnā reģioselektīvi dod acetātu **134**, kuru oksidējot ar asinssāli, iegūst hinometīdu **135**. Tālāk reakcija ar metilmagnija jodīda pārākumu dod diaizvietoto spirtu **136**, kuru var pārvērst fenilacetātā **140** ar acetanhidrīdu fāzu pārneses apstākļos. Standartprocedūra, alkilējot spirtu **140** ar pikolilhlorīdu, un sekojoša aizsargrupas noņemšana dod ēteri **142**.

Ja spirtu **136** apstrādā ar mezilhlorīdu, tas veido mezilātu **137**. kurš reakcijā ar pikolimerkaptānu veido α,γ–divaizvietotu tioēteri **138**.

Lai parādītu, ka augstāk aprakstīto savienojumu rindā pastāv principiāla iespēja realizēt tiešas pārejas no jebkura savienojuma uz tā sarežģītāko (α–aizvietoto) homologu divās stadijās, tioēteri **107** oksidējām par hinometīdu **143**. kurš reakcijā ar metilmagnija jodīdu deva diaizvietoto tioēteri **139**.

4. Nikanartīna atvasinājumu bioloģiskā aktivitāte

Bioloģisko pārbaužu gaitā veikta sintezēto savienojumu sekojošu īpašību pārbaude.

* Hipolipidēmiskās darbības pārbaude, nosakot triglicerīdu veidošanās inhibēšanu ar nikotīnskābi kā standartu.
* Antioksidatīvo īpašību novērtējums, nosakot konjugēto diēnu veidošanās laiku[35] un ar tiobarbitūrskābi reaģējošo vielu (TBARS) koncentrāciju[36].
* Antiproliferatīvā efekta novērtējums, izmantojot žurkas aortas sieniņas muskuļu šūnas[37].

Salīdzinot Nikanartīnu ar tā tioanalogu **15**, var secināt, ka būtiskas atšķirības preparātu iedarbībās nepastāv. Tioēteris **15** nedaudz atpaliek pēc savas hipolipidēmiskās iedarbības, bet tā ir stabila ilgāku laiku nekā oksa-analogam **5**. Būtiski augstāka ir antiproliferatīvā iedarbība uz gludās muskulatūras sieniņām.

Metilgrupas ievešana α-stāvoklī pret fenola gredzenu nedaudz izmaina hipolipidēmisko iedarbību, to it kā paildzinot ētera **56** gadījumā. Savukārt pilnīgi neizskaidrojams ir šīs aktivitātes pilnīgs zudums tioanalogam **59**. Patlaban nav izskaidrojuma arī tikai minimālajai ietekmei uz triglicerīdu sintēzi savienojumam, kurš satur divus piridīna grupējumus - ēterim **60**. Iespējams, ka šādas parādības varētu būt saistītas ar preparātu lēno difūziju vidē.

Antioksidatīvo īpašību izmaiņa, ievadot aizvietotāju α-stāvoklī pret fenilgredzenu, pagaidām nav viennozīmīgi izvērtējama, jo novērojams būtisks diēnu veidošanās laika pieaugums, bet tikai lielās (20μM) koncentrācijās. Savukārt TBARS sintēzi šie preparāti ietekmē tāpat kā Nikanartīns. Vienīgi stirolam **58** novērojama jūtama aktivitātes samazināšanās šajā testā.

Visiem pārbaudītajiem savienojumiem šajā grupā ir augstāka antiproliferatīvā aktivitāte nekā Nikarnatīnam.

9. tabula. Ēteru bioloģiskās aktivitātes pētījumu rezultāti

Sav. Nr	Aizvietotāji R	Triglic. sint., (inh.% pret kontroli)		Konj. diēnu veid. laiks (% pret kontroli)		TBARS veid. (inh.% pret kontroli)		SMC prolif. (inh.% pret kontroli,
		240'	360'	5μM	20μM	10μM	20μM	c=50 μM)
5	R^1=R^2=R^3=H	73	76	450	-	87	90	71
15	R^1=R^2=R^3=H	60	59	360	733	90	90	98
56	R^1=Me; R^2=R^3=H	79	92	317	1600	85	86	98
55	R^1=CH_2OCH_2Py; R^2=R^3=H	-	-	267	1304	42	52	98
60	R^1=SCH_2Py; R^2=R^3=H	71	79	510	>1500	79	81	-
59	R^1=Me; R^2=R^3=H	2	5	-	-	-	-	99
58	R^1=R^2=R^3=H	78	85	-	-	36	73	98
69	R^2=Me; R^1=R^3=H	-	-	263	321	77	80	96
85	R^2=Pr; R^1=R^3=H	-	-	-	-	-	-	-
86	R^2=Bu; R^1=R^3=H	48	52	89	207	46	68	99
94	R^2=CH_2=; R^1=R^3=H	71	66	-	-	74	84	96
95	R^2= CH_2OCH_2Py; R^1=R^3=H	71	80	-	-	84	89	94
70	R^2=Me; R^1=R^3=H	67	60	237	1308	42	51	66
87	R^2=Et; R^1=R^3=H	67	71	392	614	9	62	98
88	R^2=Pr; R^1=R^3=H	43	39	354	797	40	45	98
89	R^2=Bu; R^1=R^3=H	40	64	-	-	24	45	18
118	R^3=Et; R^1=R^3=H	-	-	-	-	-	-	97
119	R^3=Pr; R^1=R^3=H	-52	41	-	-	65	70	98
120	R^3=Bu; R^1=R^3=H	-97	-28	451	922	68	83	99
107	R^3=Et; R^1=R^3=H	81	62	204	276	42	63	98
108	R^3=Pr; R^1=R^3=H	-	-	-	-	-	-	-
132	R^1=Me; R^2=Me; R^3=H	77	72	193	650	75	76	98
133	R^1=Me; R^2=Me; R^3=H	61	46	232	1527	52	60	98
142	R^1=Me; R^2=H; R^3=Pr	47	75	-	-	70	74	-
139	R^1=Me; R^2=H; R^3=Et	29	46	227	>1500	69	73	-
138	R^1=Me; R^2=H; R^3=Pr	42	43	181	466	71	74	-
127	R^1=H; R^2=Pr; R^3=Et	45	66	-	-	37	66	-

Aizvietotājs β–stāvoklī hipolipidēmisko iedarbību ietekmē minimāli, tomēr tioēteru rindā, pārejot no metilgrupas uz butilaizvietotāju, novērojama neliela aktivitātes samazināšanās. Arī šajā gadījumā otrs pikolilspirta fragments praktiski neietekmē hipolipidēmisko iedarbību (savienojums **95**).

Antioksidatīvās iedarbības tests ar TBARS norāda uz vispārēju aktivitātes samazinājumu tioēteriem, salīdzinot ar skābekļa analogiem, kas it īpaši izpaužas ar lielākiem alkilaizvietotājiem. Jādomā, ka molekulas kopējais lipofilitātes pieaugums šeit nosaka biopieejamības pazeminājumu un, līdz ar to, aktivitātes kritumu.

Negaidīts rezultāts ir praktiski jebkuras ietekmes trūkums uz šūnu proliferāciju tioēterim **89**, kamēr tā skābekļa analogs **86** uzrāda parasto aktivitātes līmeni.

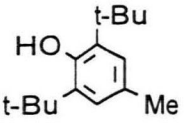
Aizvietotājs γ–stāvoklī izraisa strauju hipolipidēmiskās aktivitātes kritumu ēteriem **119** un **120**, kaut gan iespējams, ka nepieciešams ilgāks laiks piridilkarbinola atbrīvošanai no šiem savienojumiem, par ko varētu liecināt zināmas iedarbības parādīšanās propilatvasinājumam **119** pēc 360 minūtēm. Tajā pašā laikā tioēteris **107** uzrāda tādu pašu aktivitātes līmeni kā Nikanartīns, vienlaicīgi izrādoties mazāk efektīvs antioksidants, tieši tāpat kā tioēteri no iepriekšējās savienojumu grupas.

Divaizvietotu atvasinājumu rindā vairs nav izsekojamas likumsakarības starp alkilradikāļu pozīciju un aktivitātēm, bet, pieaugot preparāta molmasai, var novērot vispārēju efekta samazināšanos.

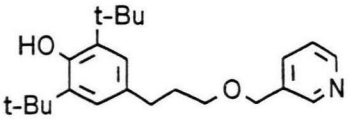
Rezumējot iegūtos rezultātus, var teikt, ka alkilaizvietotāju ievešana Nikanartīna molekulā neizmaina šī preparāta farmakoloģisko profilu, taču ļauj secināt, ka lipofilitātes pieauguma dēļ novērojama vispārēja hipolipidēmiskās un antioksidatīvās aktivitātes samazināšanās. Antiproliferatīvais efekts gandrīz visiem atvasinājumiem saglabājas konstantā līmenī.

5. Nikanartīna potenciālo metabolītu sintēze

Tā kā līdz šim literatūrā nav nekādas informācijas par Nikanartīna vai kāda cita šīs klases savienojuma metabolismu mēs, plānojot potenciālo metabolītu sintēzi, izmantojām analoģiju starp zināmo 3,5-di-*treš*-butil-4-hidroksitoluolu (**1**) un mūsu pētījuma objektu **5**:



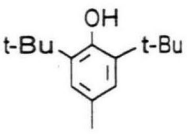
1



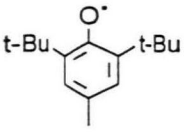
5

BHT metabolisms ir labi izpētīts, jo šo savienojumu jau kopš 1954. gada lieto kā konservantu pārtikā, kosmētikā, medicīnā un tehnikā.

Dzīvie organismi BHT pamatā metabolizē aknās, ko pierāda eksperimenti ar radioaktīvi iezīmētu preparātu[38]. Pirmais solis šī produkta metabolisko pārvērtību ciklā ir fenoksilradikāļa **144** rašanās, ko pierāda arī EPR eksperimenti[39]. Acīmredzot no šī radikāļa veidojas arī viens no galvenajiem metabolītiem: hinometīds **145**[40,41]. Tālākie metabolisma pētījumi ar pelēm pierāda[42], ka dzīvajos organismos no 3,5-di-*treš*-butil-4-hidroksitoluola rodas vesela rinda produktu: hinols **146**, benzilspirts **147**, benzaldehīds **148** un hinons **149**.



1



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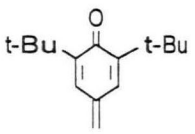
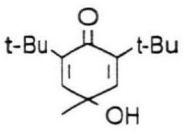
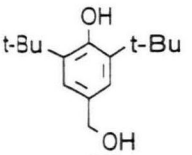


145



146



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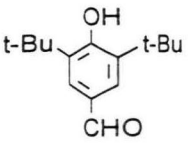
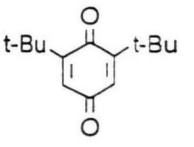


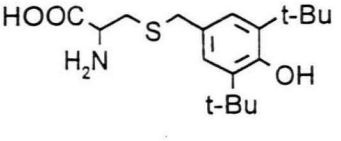
148



149

Pastāv uzskats[43], ka savienojumi **145-149** nav secīgu pārvērtību virknes sekas, bet gan, ka BHT metabolismam organismā ir vairāki alternatīvi ceļi, katrs no kuriem atbild par kāda atsevišķa produkta rašanos.

Hinometīds **145** ir ļoti reaģētspējīga daļiņa, kas ātri saistās ar nukleofiliem aģentiem, un tieši tāpēc to saista ar toksiskajiem blakusefektiem, kādi novērojami, ilgstoši barojot eksperimentālajiem dzīvniekiem 3,5-di-*treš*-butil-4-hidroksitoluolu saturošu pārtiku[44]. Pētot šos blakusefektus, jau 1979. gadā tika izteikta doma[45], ka dabiskie nukleofili: cisteīns, glutations spēj saistīties ar hinometīdu **145** un pārvērst to ūdenī šķīstošos savienojumos **150** un **151**, tādējādi atbrīvojot organismu no toksisku vielu uzkrāšanās.



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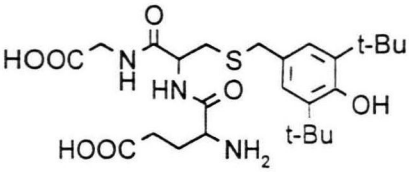
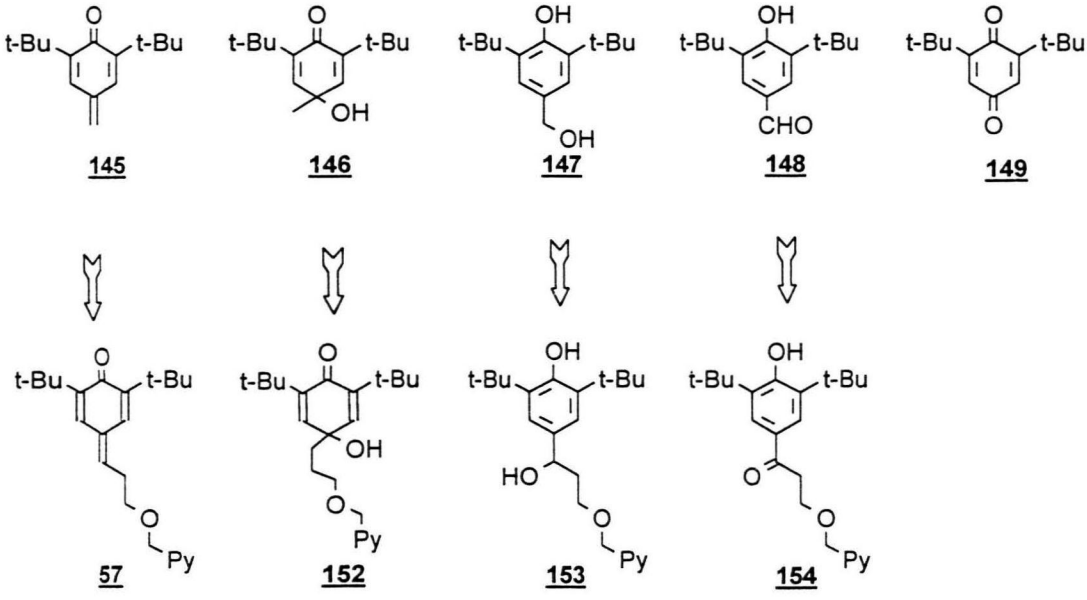
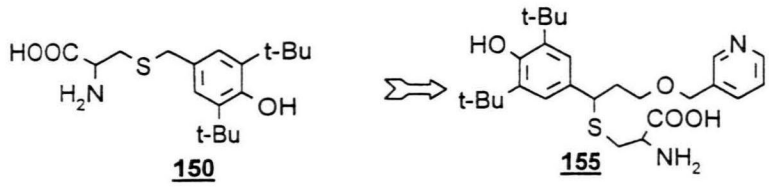


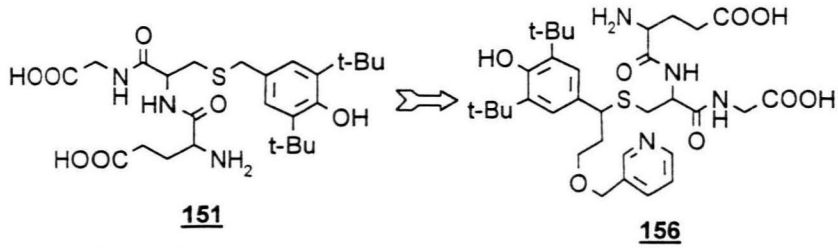
151

Tika parādīta BHT metabolītu līmeņa samazināšanās aknās, dodot dzīvniekiem ar cisteīnu un glutationu bagātinātu barību, bet pašus aduktus neizdevās izdalīt. Hinometīda **145** un glutationa reakcijas produktu 1990. gadā izdalīja cita zinātnieku grupa[43].

Apkopojot visu iepriekš izklāstīto materiālu, mēs izveidojām sekojošu shēmu, kura parāda zināmajiem BHT metabolītiem **145-151** atbilstošos analogus Nikanartīna gadījumā:

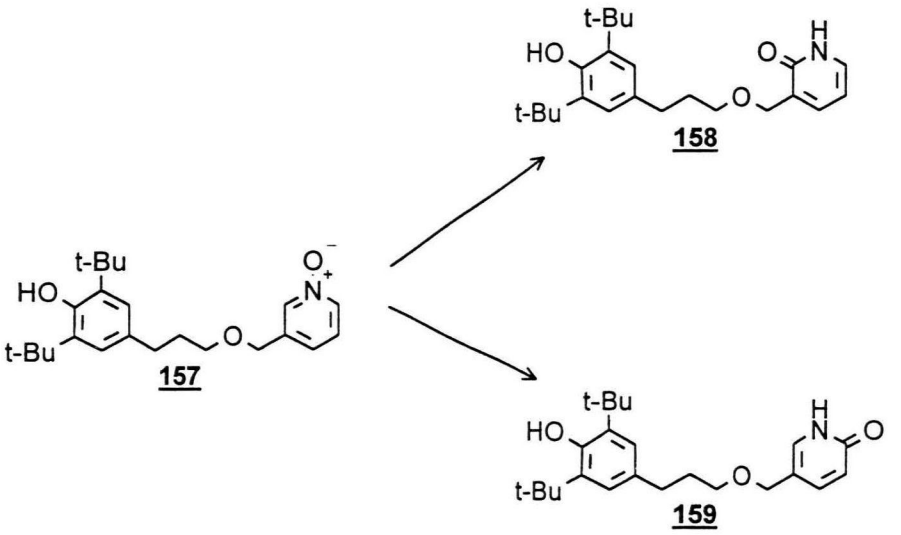




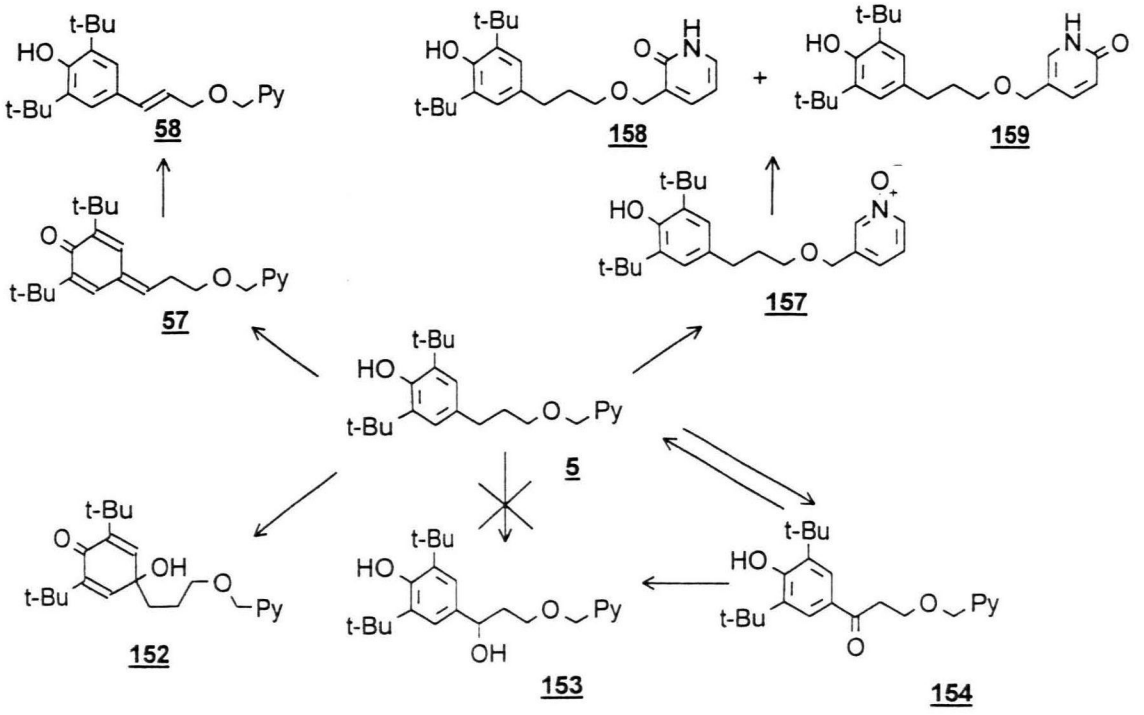


Hinometīdam **145** atbilst jau iepriekš aprakstītais Nikanartīna oksidācijas produkts **57**. Hinolu **146** un benzilspirtu **147** varētu aizvietot atbilstošie atvasinājumi **152** un **153**. Aldehīda **148** analogs ir ketons **154**. Ja metabolisko pārvērtību procesā notiek oglekļa ķēdes degradācija, gala rezultātā rodas hinons **149**, tāpēc mūsu shēmā šim savienojumam nav analoga. Savukārt cisteīna un glutationa aduktiem **150** un **151** atbilst derivāti **155** un **156**.

Pikolilspirtam **3** atbilstošās metaboliskās pārvērtības[46] reprezentē N-oksīds **157** un tā pārgrupēšanās produkti **158** un **159**.



Lai iegūtu visus nepieciešamos produktus, izvēlējāmies sekojošu sintēzes taktiku: apstrādājot Nikanartīnu ar dažādiem oksidējošiem aģentiem varētu mēģināt pagatavot tā atvasinājumus, neizmantojot garas totālās sintēzes shēmas. Hinometīdu iegūšanai jau bijām apguvuši oksidēšanu ar sarkano asinssāli baziskā vidē. Oksidācijas pa benzilisko oglekļa atomu saskaņā ar literatūru[47] var izdarīt, izmantojot halogenēšanas reakcijas. Savukārt, mijiedarbība ar peroksiskābēm ir labi pazīstama metode piridīna N-oksīdu pagatavošanai.



Apstrādājot Nikanartīnu metilēnhlorīda šķīdumā ar *m*-hlorperoksibenzoskābi ar vairāk nekā 80% iznākumu ieguvām atbilstošo N-oksīdu **157**. Literatūrā aprakstītās pārgrupēšanas ar acetanhidrīdu[48] vietā mēs izmantojām trifluoroacetilhlorīdu, kas zemā (-70°C) temperatūrā ļauj izvairīties no fenoliskās hidroksilgrupas acilēšanas arī nelietojot aizsarggrupas. Izomēri **158** un **159** radās attiecībās 1/1.

Nikanartīna oksidēšana par hinometīdu **57** aprakstīta jau iepriekš, apspriežot rezultātus par aizvietotāju ievadīšanu molekulā. Hinoīdā produkta **57** pārvēršana atbilstošajā stirolā izdodas ar >90% iznākumu, vienkārši izlaižot šķīdumu caur kolonnu ar *Kieselgel 60*. Jāatzīmē, ka šī pārvērtība neizdodas, ja izmanto mazāk aktīvo *Silasorb 600* sorbentu. Līdzīgus procesus literatūrā skaidro ar silikagela virsmas skābumu, kas ierosina aprakstītās izomerizācijas[49].

No fenola **1**, oksidējot ar benziltrimetilamonija tribromīdu, ar labu iznākumu var iegūt benzilspirtu **147**[50]. Piemērojot šo reakciju Nikanartīnam, spirtu **153** neizdevās iegūt ne ar šo reaģentu, ne ar līdzīgas iedarbības metil-3-(1,1,1-trimetilhidrazīnija) propionāta tribromīdu[51]. 1 ekvivalents oksidētāja deva 75-80% hinola **152**, kamēr tribromīda pārākumā (2 ekvivalenti) radās ketons **154**. Šī karbonilsavienojuma mijiedarbība ar litija alumohidrīdu -20°C temperatūrā ļāva iegūt vajadzīgo benzilspirtu **153**. Mēģinot reducēšanu izdarīt ar nātrija borhidrīdu istabas temperatūrā, praktiski kvantitatīvi rodas Nikanartīns. Acīmredzot bāziskajā vidē, ko rada nātrija borhidrīds, no spirta **153** var veidoties fenolāts, kura hinoīdais tautomērs var reducēties ar nākamo hidrīda ekvivalentu, dodot sākotnējo fenolu **5**.

Mēģinājumi iegūt Nikanartīna un cisteīna aduktu **155** polārās neūdens vidēs, izmantojot cisteīna sāļus ar stiprām bāzēm, bija neveiksmīgi. un vienīgais izolētais produkts šajā gadījumā bija stirols **58**.

Apstrādājot hinometīda **57** šķīdumu metilēnhlorīdā ar cisteīna pārākumu ūdens šķīdumā fāzu pārneses katalizatora klātbūtnē, izdevās iegūt aminoskābi **155** ar 57% iznākumu pēc kristalizācijas no ūdens.

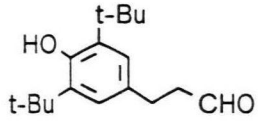
Līdzīgā veidā izdarot reakciju ar glutationu, pēc reakcijas maisījuma attīrīšanas no sāļiem uz vāji baziska jonīta ar 32% iznākumu var iegūt glutationa aduktu **156**.

Tādejādi vienā vai divās stadijās no Nikanartīna var iegūt visus tā potenciālos metabolītus, ieskaitot arī pievienošanās produktus aminoskābēm, ar labu iznākumu un neizmantojot aizsarggrupas.

5.1. Metabolītu bioloģisko pārbaužu rezultāti

Izmantojot sintezētos savienojumus kā standartvielas, izdarītas primārās toksikoloģijas studijas ar suņiem un žurkām ar mērķi noskaidrot galvenos Nikanartīna metabolisko pārvērtību produktus. Savienojumi identificēti, analizējot eksperimenta dzīvnieku urīnu, kā arī aknu un žultspūšļa audu homogenizātus ar gāzu un šķidruma hromatomasspektrometrijas metodēm un salīdzinot iegūtos datus ar standartu masspektriem.

Bez jau iepriekš minētajiem produktiem atrastas vēl sekojošas vielas:



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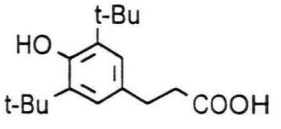
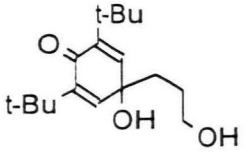
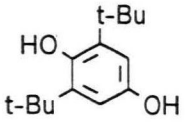


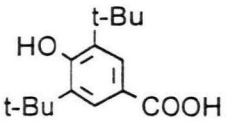
161



162



163



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Visu metabolisko studiju rezultāti apkopoti 10. tabulā.

10. tabula. Eksperimentāli atrastie Nikanartīna metabolisko pārvērtību produkti

	Studija uz suņiem	Studija uz žurkām*
Aknu homogenizāts	5, 9, 58, 145, 148, 149, 153, 154, 156, 157, 158, 159, 160, 161, 164	9, 58, 145, 153, 154, 160, 161, 162, 163, 164
Žultspūslis	5, 9, 58, 145, 148, 149, 153, 154, 156, 157, 158, 159, 160, 161, 164	nav datu
Urīns	5, 9, 58, 94,145, 148, 149, 153, 154, 156, 157, 158, 159, 160, 161, 164	zīmes no 5
Asins serums	nav datu	9, 161
Citu orgānu (kuņģa, smadzeņu, plaušu, sirds, smadzeņu, nieru) homogenizāti	nav datu	nikotīnskābe, 9, 58, 149, 152, 160, 161, 163

*- objekti analizēti tikai ar gāzu hromatomasspektrometrijas metodi.

Kā redzams no 10. tabulas datiem, metabolisma produktu sadalījumā nav novērojama nekāda orgānu specifitāte. Līdzās mūsu prognozētajiem metabolītiem atrasti arī molekulas degradācijas produkti, kā, piemēram, 3-(3,5-di-*treš*-butil-4-hidroksifenil)propanols **9** un tā secīgas oksidēšanās produkti: aldehīds **160** un karbonskābe **161**. Hinola **162** klātbūtne izskaidrojama vai nu ar fenola atvasinājuma **9** oksidēšanos, vai ar hinola **152** sabrukšanu. Saprotama ir arī alkilķēdes noārdīšanās produktu - aldehīda **148**, karbonskābes **164**, hinometīda **145**, hinona **149** tam atbilstošā hidrohinona **163** atrašanās pētāmajos objektos.

Neskatoties uz to, ka līdz šim nav pierādīta hinometīda **57** atrašanās starp metabolisma produktiem, glutationa atvasinājuma **156** izdalīšana no metabolisma produktiem norāda, ka organisma atbrīvošana no aktīvajām hinometīda molekulām arī Nikanartīna gadījumā var risināties ūdenī šķīstošu konjugātu sintēzes ceļā. Visos objektos ir atrasts arī stirols **58**, kas arī netieši liecina par hinometīda kā reaktīva starpprodukta eksistenci preparāta **5** metaboliskajās pārvērtībās.

Rezumējot šo nodaļu, jāsecina, ka sākotnēji izvirzītā ideja par Nikanartīna metabolītu prognozēšanu, izmantojot literatūras analoģijas par 2,6-di-*treš*-butil-4-metilfenola oksidatīvo metabolismu un kombinējot tās ar labi izpētītajām pridilkarbinola (**3**) pārvērtībām, izrādījusies pareiza.

Tāpat sevi pilnībā attaisnojusi sintēzes taktika - modelēt metaboliskās pārvērtības, izmantojot dažādus oksidējošus aģentus reakcijās ar Nikanartīnu. Visi produkti, tai skaitā arī aminoskābju atvasinājumi, iegūti vienas līdz divu stadiju rezultātā.

6. Secinājumi

1. Ar Viljamsona ēteru sintēzes metodi dimetilsulfoksīdā nātrija sārma klātbūtnē parādīts, ka iespējama 2,6-di-*treš*-butil-4-[5-(3-piridil)-4-oksapentil]fenola (Nikanartīns) un tam analogu sazarotas virknes ēteru un tioēteru sintēze, neizmantojot aizsargrupas pie fenoliskās hidroksilgrupas.

Noskaidrots, ka aizvietotājs α–stāvoklī pret fenola gredzenu neietekmē, bet alkilaizvietotājs β–stāvoklī pret fenola gredzenu apgrūtina ēteru kondensācijas reakciju un samazina iznākumus.

No otrējiem spirtiem atvasināti mezilāti (aizvietotājs γ–stāvoklī pret fenola gredzenu) vispār nedod ēterus reakcijā ar pirildil-3-karbinolu, taču reaģē ar piridil-3-merkaptānu, veidojot atbilstošos tioēterus.

2. Izstrādāta universāla metode (3,5-di-*treš*-butil-4-hidroksifenil)metil-(3-piridil)alkilēteru sintēzei no atbilstošajiem 1-[(3,5-di-*treš*-butil-4-hidroksifenil)metil]-(3-oksialkil)piridīnija sāļiem pārgrupēšanās reakcijā nātrija hidrīda ietekmē. Pārgrupēšanās notiek caur 2,6-di-*treš*-butil-4-metilēn-cikloheksa-2,5-diēnona (hinometīda) intermediātu. Reakcijas iznākumu neiespaido piridilalkanola alkilķēdes garums.

3. Balstoties uz 1-[(3,5-di-*treš*-butil-4-hidroksifenil)metil]-(3-alkiloksi)piridīnija sāļu pārgrupēšanās reakciju, izstrādāts jauns aģents (3,5-di-*treš*-butil-4-hidroksifenil)metilgrupu ievešanai molekulā - 1-(3,5-di-*treš*-butil-4-hidroksifenil)metil-piridīnija hlorīds, kas ļauj ar labu iznākumu iegūt malonestera atvasinājumus, kā arī sintezēt atbilstošos ēterus reakcijā ar O-trimetilsilil-(3-piridil)alkanoliem.

4. Oksidējot ar kālija ferricianīdu bāziskā vidē, no 2,6-di-*treš*-butil-4-[5-(3-piridil)-4-oksapentil]fenola (Nikanartīns) un (3,5-di-*treš*-butil-4-hidroksifenil)metil-(3-piridil)metilētera iegūti stabili hinometīdi, kuri reakcijās ar oglekļa, skābekļa un sēra nukleofiliem dod stabilus pievienošanās produktus.

2,6-Di-*treš*-butil-4-[5-(3-piridil)-4-oksapentil]fenolam atbilstošais hinometīds ir universāls sintons α-stāvoklī pret fenola gredzenu aizvietotu Nikanartīna analogu iegūšanai.

5. Parādīts, ka 3,5-di-treš-butil-4-hidroksibenzaldehīda metil-(3-piridil)metil- un di-(3-piridil)metilacetālu oksidēšanā ar kālija ferricianīdu dominējošie ir pārgrupēšanās produkti - 2,6-di-treš-butil-4-(3-piridil)metilcikloheksa-2,5-diēnon-4-karbonskābes metil- un (3-piridil)metilesteri attiecīgi. Pretstatā tam atbilstošā dimetilacetāla un agrāk aprakstītās benzaldehīda acetālu oksidēšanas reakcijas produkti ir benzoskābju esteri.

6. Atrasts oriģināls paņēmiens lipofīlas molekulas - Nikanartīna un ūdenī šķīstošā glutationa addukta veidošanās dzīvnieku organismā modelēšanai ķīmiskā eksperimentā starpfāzu pārneses katalīzes apstākļos. Savienojums iegūts tiešajā reakcijā starp glutationu un no Nikanartīna pagatavoto hinometīdu. Analoģiski sintezēts cisteīna pievienošanās produkts.

7. Izmantojot dažādus oksidējošos aģentus, selektīvās ķīmiskajās reakcijās tieši no Nikanartīna sintezēti tā potenciālie metabolīti, kuru veidošanās *in vivo* toksikoloģijas eksperimentos uz suņiem un žurkām pierādīta ar hromatomasspektrometrijas metodi.

7. Eksperimentālā daļa

^{1}H PMR spektri uzņemti uz Bruker 90MHz aparāta. Kušanas punkti noteikti uz *Boetius* kušanas galdiņa un nav koriģēti. Reakcijas izdarītas argona atmosfērā, reaģenti un šķīdinātāji sagatavoti kā aprakstīts literatūrā [52]

Dietil-2-(3,5-di-*treš*-butil-4-hidroksifenil)metilmalonāts **31**

500mg 75% nātrija hidrīda (15.6mmol) suspendē 50ml sausa dimetilsulfoksīda un maisot argona atmosfērā pievieno 2.4ml (15.8mmol) malonestera. Pēc gāzu izdalīšanās beigām iegūtajam dzidrajam šķīdumam 30min. laikā piepilina 5.0g (15.0mmol) 1-(3,5-di-*treš*-butil-4hidroksifenil)metilpiridīnija hlorīda **29** šķīdumu 150ml sausa dimetilsulfoksīda. Reakcijas maisījumu turpina maisīt istabas temperatūrā vēl 1h, tad izlej 500ml ūdens un ekstrahē ar ēteri (3x250ml). Apvienotos ētera ekstraktus mazgā ar 5% sālsskābes šķīdumu un pēc tam ar piesātinātu nātrija hlorīda šķīdumu, žāvē uz nātrija sulfāta un ietvaicē vakuumā. Iegūst 5.55g (14.7mmol, 97.8%) viskozas eļļas, ko attīra destilējot *Kugelrohr* aparātā. Iznākums 5.48g (14.49mmol, 96.6%).

^{1}H PMR ($CDCl_3$, TMS, δ): 1.20(6H, t, J=7.0Hz, 2x$COOCH_2\underline{CH_3}$); 1.42(18H, s, 2xt-Bu); 3.13(2H, m, CH_2Ar); 3.60(1H, dd, J_1=7.6Hz, J_2=7.1Hz, $CH(COOEt)_2$); 4.16(4H, q, J=7.0Hz, 2x$COOCH_2$); 5.07(1H, s, ArOH); 6.98m.d.(2H, s, C_6H_2).

2,6-Di-*treš*-butil-4-[1-(3-piridil)-2-oksapent-3-il]fenols **35**

10ml 1.5M etilmagnija bromīda šķīdumam THF argona atmosfērā 0^{O}C temperatūrā 20min. laikā pievieno 1g (3.07mmol) hinometīda **33** šķīdumu 20ml tetrahidrofurāna. Pēc pievienošanas reakcijas maisījumam ļauj sasilt līdz istabas temperatūrai un maisa vēl 1h. Pēc tam uzmanīgi izlej 200ml piesātināta K,Na tartrāta šķīduma un ekstrahē ar

etilacetātu (3x50ml). Ekstraktu žāvē un ietvaicē līdz sausam. Iegūst 1.0g (96%) ētera **35**. Attīra kristalizējot no heksāna, $t_{kuš.}$=114-116^{O}C.

^{1}H PMR ($CDCl_3$, TMS, δ): 0.91(3H, t, J=7.0Hz, $CH_2\underline{CH_3}$); 1.44(18H, s, 2xt-Bu); 1.60-1.90(2H,m, $\underline{CH_2}CH_3$) 4.11(1H, m, OCHAr); 4.35(2H, d, J=6.8Hz, CH_2Py); 5.16(1H, s, ArOH); 7.04 (2H, s, C_6H_2); 7.13-7.31(1H, m, Py-5H); 7.62(1H, m, Py-4H); 8.49m.d.(2H, m, Py-2H un Py-6H).

3,5-Di-*treš*-butil-4-hidroksibenzaldehīda etil-(3-piridilmetil)acetāls **37**

1g(3.07mmol) hinometīda **33** šķīdina 20ml absolūta etanola un pievieno ≈200mg Amberlyst 15 jonīta. Šķīduma spilgti dzeltenā krāsa izzūd apmēram 0.5h laikā. Reakcijas maisījumu atstāj maisīties istabas temperatūrā pa nakti. Nofiltrē jonītu, šķīdumu ietvaicē. Iegūst 1.05g bezkrāsainu kristālu, kurus attīra kristalizējot no izopropilspirta. Iznākums: 0.98g (89%) bezkrāsainu kristālu, $t_{kuš.}$=141-143^{O}C.

^{1}H PMR ($CDCl_3$, TMS, δ): 1.23(3H, t, J=6.8Hz, $CH_2\underline{CH_3}$); 1.42(18H, s, 2xt-Bu); 3.61(2H,m, CH_2O) 4.53(2H, s, $PyCH_2$); 5.12(1H, s, ArOH); 5.50(1H, s, OCHO); 7.20(3H, m, Py-5H un C_6H_2); 7.66(1H, m, Py-4H); 8.44m.d.(2H, m, Py-2H un Py-6H).

1-O,2-O,3-O,4-O-Diizopropilidēn-6-O-[1-(3,5-di-*treš*-butil-4-hidroksifenil)-2-oksa-3-(3-piridil)propil]-α-D-galaktoze **39**

1g(3.07mmol) hinometīda **33** šķīdina 15ml absolūta acetonitrila un pievieno ≈200mg Amberlyst 15 jonīta. Šķīdumam pievieno 0.78g (0.003mmol) diizopropilidēn-galaktozes, kura izšķīdināta 10ml acetonitrila. Maisījuma spilgti dzeltenā krāsa izzūd apmēram 2h laikā. Maisa istabas temperatūrā pa nakti. Nofiltrē jonītu, šķīdumu ietvaicē. Iegūst 1.80g viskozas eļļas, kuru sakristalizē, apstrādājot ar

heksānu. Attīra kristalizējot no heksāna. Iznākums: 1.60g (91%) bezkrāsainu kristālu, $t_{kuš.}$=105-107^{O}C.

^{1}H PMR ($CDCl_3$, TMS, δ): 1.20-1.58(30H, m, 2x$(CH_3)_2$C un 2xt-Bu); 3.69-4.71(6H, m, galaktozes CH); 4.64(2H, s, CH_2Py); 5.22(1H, s, ArOH); 5.53(1H, d, J=5.0Hz, galaktozes glikozīdiskais-CH); 5.61(1H, s, OCHO); 7.11-7.36(3H, m, Py-5H un C_6H_2); 7.69(1H, m, Py-4H); 8.40-9.64m.d. (2H, m, Py-2H un Py-6H).

2,6-Di-*treš*-butil-4-[1-dietoksikarbonilmetil-5-(3-piridil)-4-oksa–pentil]fenols **61**

250mg 75% nātrija hidrīda (7.8mmol) suspendē 50ml sausa dimetilsulfoksīda un, maisot argona atmosfērā, pievieno 1.2ml (7.9mmol) malonestera. Pēc gāzu izdalīšanās beigām iegūto dzidro šķīdumu uzsilda līdz 90^{O}C un pa pilienam pievieno 2.6g (7.4mmol) hinometīda **57** šķīdumu 15ml ētera ar tādu ātrumu, lai viss ēteris pievienošanas laikā iztvaikotu. Reakcijas maisījumu izlej 500ml ūdens un ekstrahē ar ēteri (3x100ml). Apvienotos ētera ekstraktus mazgā ar piesātinātu nātrija hlorīda šķīdumu, žāvē uz nātrija sulfāta un ietvaicē vakuumā. Iegūto eļļu šķīdina heksāna / etilacetāta maisījumā (1:1) un hromatografē uz 300x25mm kolonnas ar *Kieselgel* 60. Ietvaicējot produktu saturošās frakcijas iegūst 2.6g bezkrāsaina kristāliska **61**, kuru attīra, kristalizējot no izopropilspirta. Iznākums: 2.3g (4.5mmol, 60.7%)

^{1}H PMR ($CDCl_3$, TMS, δ): 0.89(3H, t, J=7.5Hz, $COOCCH_3$); 1.27(3H, t. J=7.5Hz, $COOCCH_3$); 1.39(18H, s, 2xt-Bu); 1.73-2.18(2H, m, CCH_2C); 3.31(2H,m, CH_2O); 4.52(1H, dt, J_1=11Hz, J_2=15Hz, ArCH); 3.84(2H, q, J=7.5Hz, $COOCH_2$); 4.20(2H, q, J=7.5Hz, $COOCH_2$); 4.22(1H, d, J=15Hz, HC)$COOEt)_2$); 4.27(2H, s. CH_2Py); 5.04(1H, s, ArOH); 6.93(2H, s. C_6H_2); 7.09-7.33(1H, m, Py-5H); 7.58(1H, m, Py-4H); 8.38-8.56m.d. (2H, m, Py-2H un Py-6H).

2,6-Di-*treš*-butil-4-[1-metoksi-5-(3-piridil)-4-oksa–pentil]fenols **64**

1g(2.84mmol) hinometīda **57** šķīdina 20ml absolūta metanola un pievieno ≈200mg Amberlyst 15 jonīta. Šķīduma spilgti dzeltenā krāsa izzūd apmēram 0.5h laikā. Reakciju atstāj maisīties istabas temperatūrā pa nakti. Nofiltrē jonītu, šķīdumu ietvaicē. Iegūst 0.98g (2.56mmol, 89%) produkta **64** kā bezkrāsainus kristālus. Kristalizējot no heksāna, produkts pamazām pārvēršas stirolā **58**.

^{1}H PMR ($CDCl_3$, TMS, δ): 1.43 (18H, s, 2xt-Bu); 1.82-2.20(2H,m, CCH_2C) 3.18(3H, s, OCH_3); 3.40-3.73(2H, m, CH_2O); 4.20(1H, m, OCHAr); 4.50(2H, s, $PyCH_2$); 5.13(1H, s, ArOH); 7.04(2H, s, C_6H_2); 7.16-7.36(1H, m, Py-5H); 7.55-7.73(1H, m, Py-4H); 8.44-8.61 m.d.(2H, m, Py-2H un Py-6H).

2,6-Di-*treš*-butil-4-[1-metilamino-5-(3-piridil)-4-oksa–pentil]fenols **65**

1g(2.84mmol) hinometīda **57** šķīdina 10ml sausa tetrahidrofurāna un ievieto biezsienu ampulā. Turpat pievieno ≈10ml šķidra metilamīna. Ampulu aizkausē un atstāj istabas temperatūrā uz 12h. Pēc tam šķīdumu ietvaicē. Iegūst 1.10g produkta **65,** kuru mazgā ar heksānu un filtrē. Iznākums: 0.94g (2.45mmol, 86%). Kristalizējot no heksāna, produkts pamazām pārvēršas stirolā **58**.

^{1}H PMR ($CDCl_3$, TMS, δ): 1.42 (18H, s, 2xt-Bu); 1.67-2.18(2H,m, CCH_2C) 2.29(3H, s, NCH_3) 3.20-3.67(3H, s, CH_2O un NCHAr); 4.46(2H, s, $PyCH_2$); 5.11(1H, bs, ArOH); 7.02(2H, s, C_6H_2); 7.24(1H, m, Py-5H); 7.62(1H, m, Py-4H); 8.49m.d.(2H, m, Py-2H un Py-6H).

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1. Pielikums

Synthesis and biological activity of 2,6-di-*tert*-butyl-4-[5-(3-pyridyl)-4-oxa-pentyl]phenols and their *thia* analogues.

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Abstract. Convenient large scale procedure for the synthesis of *Nicanartine* (2,6-di-tert-butyl-4-[5-(3-pyridyl)-4-oxapentyl]phenol), an antiatherosclerotic compound, was developed. Synthesis of *thia* analogue as well as various branched-chain *oxa-* and *thia-* derivatives of the parent compound were investigated and 25 compounds were obtained. Screening of compounds in 4 different biological tests revealed an antiatherosclerotic profile similar to that of *Nicanartine*.

Keywords: antiatherosclerotic activity / antioxidants / butylated hydroxytoluene / pyridyl-3-carbinol.

Introduction.

Atherosclerosis is the principal cause of heart attack, stroke and gangrene of the extremities. It is responsible for *c.a.* 50% of all mortality in most industrial countries.

Recently Ross[1] proposed the "response-to-injury" hypothesis of atherogenesis. According to this different risk factors lead to endothelial dysfunction. Evidence is found to suggest that oxidatively modified low- density lipoproteins (*ox-LDL*) is a key component in endothelial injury[2]. Being formed by the endothelium, *ox-LDL* may directly injure itself initiating the adherence and migration of leukocytes into the subintimal space. Uptake of *ox-LDL* will lead to foam cell formation[3] and, among others, may alter the synthesis of growth-regulatory molecules. As a consequence arises the stimulation of the migration and proliferation of smooth muscle cells (*SMC*) into the subintimal space causing the life-threatening narrowing of arteries.

Searching for a drug, which might address therapeutically some of the most important aspects of atherogenesis, Gold et al.[4] have synthesized new BHT-ethers demonstrating their potential use as antiatherosclerotic drugs.

The mentioned class of compounds and namely *Nicanartine* (2,6-di-tert-butyl-4-[5-(3-pyridyl)-4-oxapentyl]phenol, A1, Mrz 3/124, Fig.1) have been shown to act as lipid lowering agents, inhibitors of LDL-oxidation and SMC-proliferation[5]. Moreover, the action of *Nicanartine* on balloon-katheter-induced restenosis (personal comm.) and PAI-1 production *in vitro* and *in vivo* has been demonstrated[6].

Here we wish to report a convenient large scale method for the synthesis of *Nicanartine*, instead of the 6 step procedure, elaborated by Gold and

coworkers[4], and, moreover, the synthetic route to the corresponding *thia* analogue A2 as well as series of branched chain derivatives of both A1 and A2 designed to evaluate the influence of alkyl substituents on both lipid-lowering and antioxidant properties of compounds obtained (Fig. 1). According to our hypothesis, an substituent at C1 atom (type A, fig.1) might affect the antioxidative properties of the parent compound, whereas a chain branch at C3 (type C, fig.1) should hinder the cleavage of the ether bond, making thus the release of pyridyl carbinol and, correspondingly, lipid lowering activity, more prolonged. An alkyl radical at C2 (type B, fig.1) was designed to help us to evaluate the inflence of increase in lipofility of the *Nicanartine* analogues on their biological action.

Chemistry.

We started the synthesis of Mrz 3/124 (A1) from commercially available methyl 3-(3,5-di-tert-butyl-4-hydroxyphenyl)propionate (Ionox 520, (2), scheme 1):

Reduction of ester 2 *via* $NaBH_4$ method[7] gave alcohol 3 in high yield. Treatment of 3 with mesyl chloride afforded mesylate 4. Williamson's synthesis[8] in DMSO led to the ether A1 in 75% yield (see table). The total yield of Mrz 3/124 was *c.a.* 60%. This method turned out to be highly appropriate also for a large scale synthesis (up to 500g of ester 2). Mesylate 4 in the reaction with picolyl mercaptan generated *in situ* from picolylthiouronium salt[9] and NaOH gave corresponding thioether A2 (see table).

There are several possibilities to obtain further type A derivatives of Mrz 3/124 (scheme 2).

On the one hand reaction of 2,6-di-*tert*-butylphenol (5) with α,β-unsaturated compounds could afford the substituent at C1 atom. Thus addition of dimethyl

maleate to the phenol 5, followed by reduction of *bis*-ester obtained and subsequent treatment with mesyl chloride, gave *bis*-mesylate 8b (R^1=CH_2OMs), which was converted to *bis*-ether A4 (see table). However, both availability and reactivity of unsaturated compounds as well as rigorous reaction conditions are limitations of this method.

Another approach suggests utilization of quinone methide systems in reactions with nucleophiles[10]. Alcohol 3 was regioselectively acetylated with Ac_2O in pyridine and oxidized by common procedure[11,12,13] to give quinone methide 10 as a bright yellow crystalline product. The intermediate 10 was stable enough to be crystallized from hexane. Treatment of quinone methide 10 with an excess of methylmagnesium iodide in ether at reflux, followed by reaction with mesyl chloride, afforded mesylate 8a (R^1=Me), which was converted either to ether A2 or corresponding thia analogue A6 (see table). Quinone methide pathway allowed also to use ether Mrz 3/124 as a starting material for the preparation of type A derivatives of 2,6-di-tert-butyl-4-[5-(3-pyridyl)-4-oxa-pentyl]phenol. Oxidation with potassium ferricyanide in alkaline media[11] gave quinone methide 11 as a bright yellow oil. Subsequent reaction with picolyl mercaptan afforded *bis*-ether A5 (see table). However, isomerization of 11 to a styrene derivative 12, not encountered in case of quinone methide 10, always took place and is a serious drawback of this procedure.

Type B analogues of Mrz 3/124 were prepared *via* C2-substituted mesylates 18. Either alkylated malonic esters or methyl methacrylate were building blocks to introduce the substituent at C2 atom (scheme 3).

Introduction of the BHT part into malonic esters is the key step, when using this pathway for the synthesis of B type analogues of Mrz 3/124. Recent report

showed quaternary ammonium salts as 3,5-di-*tert*-butyl-4-hydroxybenzylating agents superior to corresponding halides[14]. We have introduced pyridinium salt 13 providing benzylation under mild conditions to give diesters. Hydrolysis and subsequent decarboxylation gave carboxylic acids 16b-d. Reduction afforded alcohols 17b-d easily convertible to mesylates 18. Intermediates, as diesters 14, dicarboxylic acids 15 and carboxylic acids 16, were used crude, except analytical samples, and flash chromatography took place only for isolation of alcohols 17b-d.

Alternatively, treatment of phenol 5 with methyl methacrylate, followed by reduction of ester 21a obtained and reaction with mesyl chloride, gave mesylate 18a (R^2=Me). However, reaction of salt 13 with alkylated malonic esters required milder conditions, and various substrates are readily available. Condensation of mesylates 18a-d under the same conditions as described for 4 gave both ethers B1-B4 and thio-analogues B7-B10 (see table), however the yields were lower and flash chromatography was used for isolation of products. Similar scheme allowed to introduce the second pyridine moiety at C2. Alkylation of malonic ester or methylmalonic ester, followed by reduction and treatment with mesyl chloride, gave corresponding *bis*-mesylates 20a-b. However, condensation with pyridylcarbinol gave mixture of products B5 and B6 if R^2=H (see table). Introduction of methyl group (R^2=Me) excluded elimination and cyclobutane derivative 21 was the main product.

The introduction of the substituents at C3 atom to obtain type C analogues of Mrz 3/124 was achieved *via* ketones 26a-c (scheme 3). Hydrolysis of commercially available *Ionox-520* (2) and following treatment with thionyl chloride gave acyl chloride 24. Interaction with dimethylamine gave

corresponding amide 25, which was refluxed with Grignard reagents[17] to give ketones. However, the reaction proceeded very slowly (170h at room temperature for 26c) and the yields were quiet moderate. Alternatively, one can prepare ketones using acyl halides and Grignard reagents in the presence of ferric acetylacetonate[15,16]. Reaction of the acyl chloride 24 with 2 eq. of Grignard reagents gave corresponding ketones. Yield was nearly quantitative in case of propyl ketone 26b, whereas only 53% were achieved for ethyl derivative 26a. Obviously, the purity of acyl chloride 16 is of great importance in this procedure; however, the purification of 24 caused problems due to the self acylation. Moreover, scaling up decreased the yields of this method dramatically. Obviously, dimethylamine method should be preferred in large scale (25g and more) syntheses.

Reduction of ketones, followed by treatment with mesyl chloride, afforded mesylates 28a-b. Condensation with picolyl mercaptan gave expected ethers C4,C5 (see table); however, this method was fully inappropriate for the preparation of oxa-analogues due to elimination. That is why protected alcohols 31a-c became the key intermediates. Known procedure[4] (method A) includes reduction of ketone, THP protection of aliphatic OH group and treatment with Ac_2O under phase transfer conditions (PTC), followed by removal of THP protection. In our hands it gave only 37% total yield of propyl alcohol 31b. Compared to this, direct acetylation (method B) of alcohols 27a and 27c gave 62% and 71% of desired ester respectively, however, after chromatographic isolation of the products. Alternatively (method C), acetylation of starting ketones 26a-c, followed by reduction at low temperature, resulted in 81-84% total yield of

alcohols 31a-c. Alkylation of 20a-c with picolyl chloride under PTC, followed by deprotection with $LiAlH_4$ in refluxing THF, gave ethers C1,C3 (see table).

Whether carboxylic acid 16 (for types BC and AB) or alcohol 27b (for type AC) were appropriate starting materials for miscellaneous type derivatives of Mrz 3/124 (scheme 5).

Carboxylic acids 16a,b were appropriate starting materials for the synthesis of type BC analogues of Mrz 3/124. Treatment of 16b with thionyl chloride in hexane, followed by reaction with dimethylamine, gave N,N-dimethylamide 34, which reacted with Grignard reagent to ethyl ketone[17] 35 and gave disubstituted alcohol 36 after reduction. Protection under PTC with Ac_2O afforded selectively product 37 in 70% yield. This was alkylated under PTC with excess of picolyl chloride and deprotected by $LiAlH_4$ in boiling THF to give ether BC (see table).

On the other hand, reduction of 16a, followed by regioselective acetylation, oxidation, reaction with methylmagnesium iodide and treatment with mesyl chloride, gave mesylate 39. Reaction with either pyridylcarbinol or picolyl mercaptan afforded products AB1 and AB2 (see table), respectively.

Regioselective acetylation of alcohol 27b with Ac_2O in pyridine, followed by oxidation and reaction with methylmagnesium iodide, afforded disubstituted alcohol 45. Protection under PTC (38%), followed by alkylation with picolyl chloride and deprotection, yielded ether AC1. On the other hand, alcohol 45 was converted to mesylate 46 and gave thioether AC3 (see table) by treatment with picolyl mercaptan. Alternatively, direct oxidation of ethyl derivative C4 and treatment of resulting quinone methide 49 with methylmagnesium iodide gave ether AC2 (see table).

Biology.

The lipid lowering properties of the preparations were evaluated with nicotinic acid as a standard. The antioxidative action of test compounds was estimated by two methods: the determination of lag phase for the formation of conjugated dienes[18] and determination of thiobarbituric acid reactive substances[19]. Antiproliferative effect of substances obtained was evaluated on Wistar-rat aorta smooth muscle cell (*SMC*) proliferation[20]. The test results of lipid-lowering, anti-oxidative and anti-proliferative properties of the compounds are summarized in table.

Comparison of *Nicanartine* (A1) with its *thia*-analogue A2 revealed no obvious difference. Regarding to its anti-proliferative activity A2 seemed to be more active. On the other hand, due to the very steep concentration-response relationship this compound lost activity as soon as the concentration was <50μM. This is an effect which could also be observed for the parent substance (A1).

In general, the introduction of a chain branch at C-1 atom (derivatives of type A, namely A3, A4, A6 and 12) did not result in any improvement of pharmacological properties. Moreover, A6 did not show any lipid-lowering effect, whereas A2 bearing one methyl substituent less, showed the same or even higher activity as the parent compound A1. Although this could be explained by too low vehicle control value, the reason of inactivity might be related to the structure of these compounds as well.

Similarly substituent at C-2 atom (derivatives B1, B4-B10) did not change the pharmacological profile of the compounds in comparison to the *Nicanartine* (A1). Surprisingly B6, bearing two nicotinic acid equivalents, was not superior to the other substances in the test on lipid lowering properties. Another interesting result

is the complete loss of activity for B10 in the test on SMC-proliferation. Although B10 is very similar to B4 with regard to *thia*-ether only, the *oxa*-derivative B4 showed the usual profile while B10 did not. No explanation can be given for this so far.

Very heterogeneous results were obtained with the type C derivatives of *Nicanartine* (A1). On the one hand, C2 and C3 did not reveal any lipid-lowering activity, whereas C4 was comparable with A3. In the case of C2, at least partially, a low vehicle control is responsible for the observed increase in triglycerides.

The introduction of two substituents in the molecule also did not cause any significant changes in pharmacological profile. Irrespective on the position of substituents no important differences from the parent compound (A1) were obtained.

Discussion.

Finally, we have to conclude, that the Williamson's synthesis from corresponding mesylates and pyridyl-3-carbinol is the most appropriate method for the preparation of *Nicanartine* and its analogues. Obviously, sterical hindrances of the phenolic OH group by the bulky *o*-substituents ensure regioselectivity in this reaction and there is no need for protective groups. Secondary mesylates undergo elimination under the same conditions, whereas usage of stronger nucleophile - 3-picolyl mercaptan provided corresponding *thio*-ethers.

Quinone methide systems are attractive starting materials for the substitution of Mrz 3/124 molecule at C1 atom. Both picolyl ethers and acetylated 3-(3,5-di *tert*-butyl-4-hydroxyphenyl)propan-1-ols are easily oxidized by potassium ferricyanide

to obtain stable quinone methides. Reaction with whether thiol or Grignard reagent provides substitution in high yield.

1-(3,5-Di-*tert*-butyl-4-hydroxybenzyl)pyridinium chloride is a highly versatile agent for the introduction of the BHT residue into malonic esters.

In general, one should conclude that both the *thia*-analogue of *Nicanartine* (A2) as well as the branched chain derivatives showed biological activities matching those of parent compound (A1). In fact no differences were observed in biological action of the preparations, bearing substituents at C1, C2 or C3 atoms (types A, B and C, fig.1). However, the introduction of two chain branches resulted in decrease of both antioxidative and lipid lowering activity, irrespective of the position of alkylradicals. This allows us to suggest, that the increase of lipophilicity of the compounds could be responsible for the changes of biological activity.

Some unexpected results obtained with alkylated analogues of *Nicanartine* could not be unambiguously attributed whether to the structure of compounds or vehicle control rates.

Supplementary Material Available.

General experimental procedures, yields and physical constants of the compounds described, ^{1}H NMR spectra of the syhthesized products and intermediates, HPLC conditions for the end products are given on 16 pages. Ordering information is given on any current masthead page.

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N°	# of cmpd	R	X	M.P., (°C)	Yield, (%)	Triglicerides, (inhib.% *vs* control)		Lag time of dienes, (% *vs* control)		TBARS form. (inhib.% *vs* control)		SMC prolif. (inhib.% *vs* control, c=50 μM)
						240'	360'	5μM	20μM	10μM	20μM	
1	A1	R^1=R^2=R^3=H	O	104-105	76	73	76	450	NT	87	90	71
2	A2	R^1=R^2=R^3=H	S	59-60	75	60	59	360	733	90	90	98
3	A3	R^1=Me; R^2=R^3=H	O		53	79	92	317	1600	85	86	98
4	A4	R^1=CH_2OCH_2Py; R^2=R^3=H	O		23	NT[a]	NT	267	1304	42	52	98
5	A5	R^1=SCH_2Py; R^2=R^3=H	O	95-6	59	71	79	510	>1500	79	81	NT
6	A6	R^1=Me; R^2=R^3=H	S	105-106	70	2	5	NT	NT	NT	NT	99
7	12[b]	R^1=R^2=R^3=H	O			78	85	NT	NT	36	73	98
8	B1	R^2=Me; R^1=R^3=H	O		47	NT	NT	263	321	77	80	96
9	B3	R^2=Pr; R^1=R^3=H	O	99-101	62	NT	NT	NT	NT	NT	NT	NT
10	B4	R^2=Bu; R^1=R^3=H	O	71-72	43	48	52	89	207	46	68	99
11	B5	R^2=CH_2=; R^1=R^3=H	O	95-96	37[c]	71	66	NT	NT	74	84	96
12	B6	R^2= CH_2OCH_2Py; R^1=R^3=H	O	75-76	19[d]	71	80	NT	NT	84	89	94
13	B7	R^2=Me; R^1=R^3=H	S	63-64	63	67	60	237	1308	42	51	66
14	B8	R^2=Et; R^1=R^3=H	S	oil	54	67	71	392	614	9	62	98
15	B9	R^2=Pr; R^1=R^3=H	S	82-83	48	43	39	354	797	40	45	98
16	B10	R^2=Pr; R^1=R^3=H	S	80-81	32	40	64	NT	NT	24	45	18
17	C1	R^3=Et; R^1=R^3=H	O	82-83	39[e]	NT	NT	NT	NT	NT	NT	97
18	C2	R^3=Pr; R^1=R^3=H	O	63-64	39[e]	-52	41	NT	NT	65	70	98
19	C3	R^3=Bu; R^1=R^3=H	O	oil	33[e]	-97	-28	451	922	68	83	99
20	C4	R^3=Et; R^1=R^3=H	S	73-74	53	81	62	204	276	42	63	98
21	C5	R^3=Pr; R^1=R^3=H	S	75-76	45	NT	NT	NT	NT	NT	NT	NT
22	AB1	R^1=Me; R^2=Pr; R^3=H	O	56-7	39	77	72	193	650	75	76	98
23	AB2	R^1=Me; R^2=Me; R^3=H	S	oil	50	61	46	232	1527	52	60	98
24	AC1	R^1=Me; R^2=H; R^3=Pr	O	oil	33[e]	47	75	NT	NT	70	74	NT
25	AC2	R^1=Me; R^2=H; R^3=Et	S	oil	68	29	46	227	>1500	69	73	NT
26	AC3	R^1=Me; R^2=H; R^3=Pr	S	78-79	63	42	43	181	466	71	74	NT
27	BC	R^1=H; R^2=Pr; R^3=Et	O	oil	54[e]	45	66	NT	NT	37	66	NT
28	NDGA[f]	-	-	-	-	NT	NT	NT	NT	95	NT	
29	Nic[g]	-	-	-	-	61	69	NT	NT	NT	NT	

[a] not tested

[b] obtained as a by-product from quinone methide (scheme 1).

[c] compound separated from B6, total coversion: 56%

[d] compound separated from B5, total conversion: 56%

[e] *total yields, yields for both condensation and deprotection see* supplementary material

[f] nordihydroguaretic acid.

[g] nicotinic acid

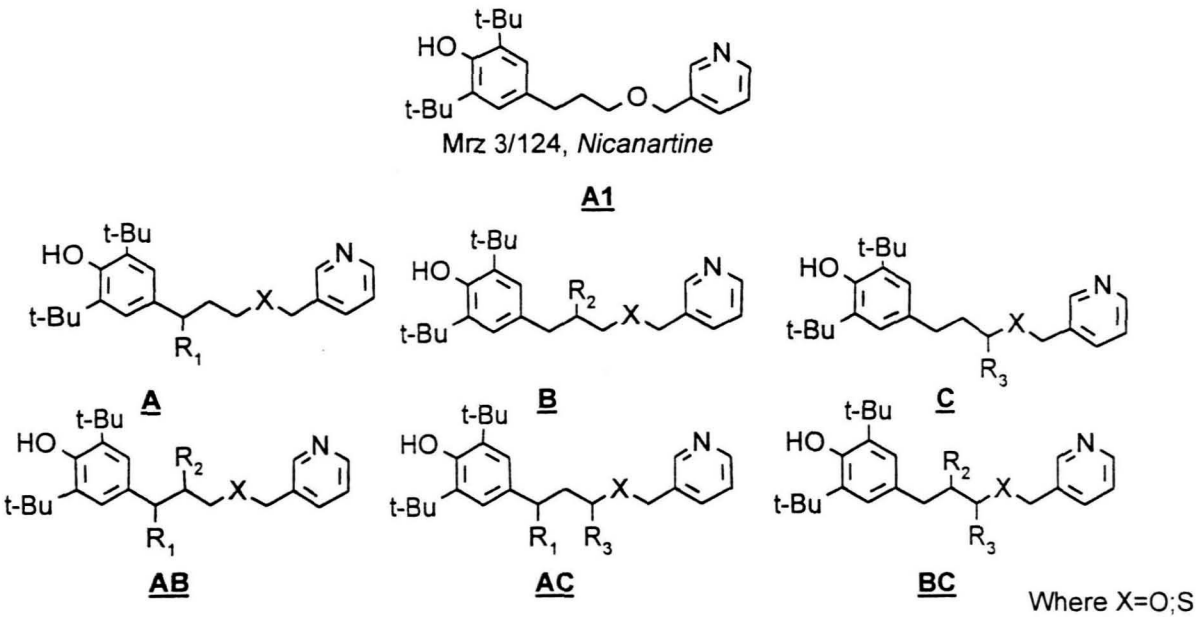
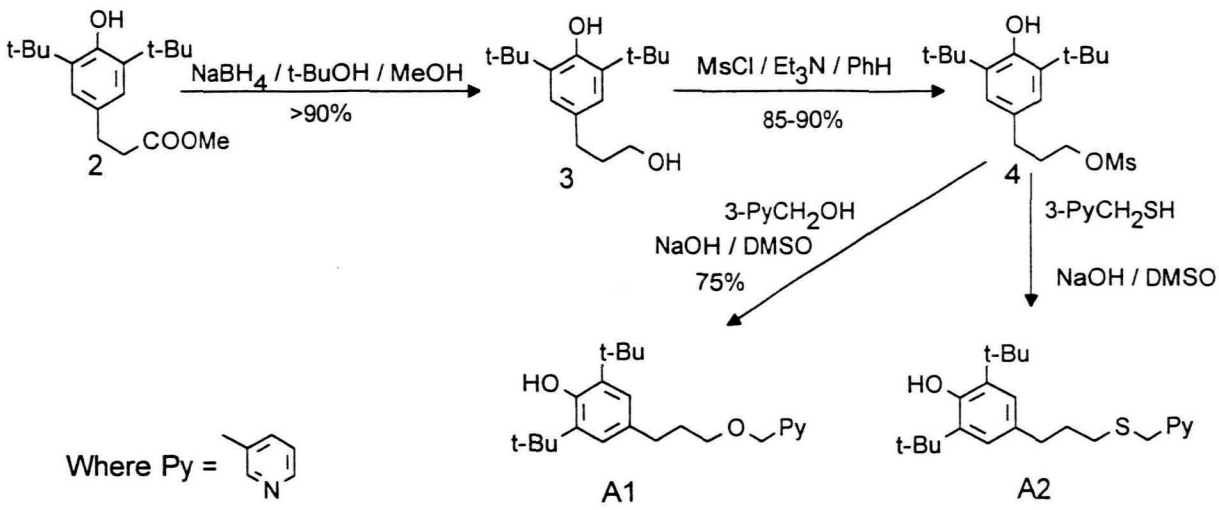
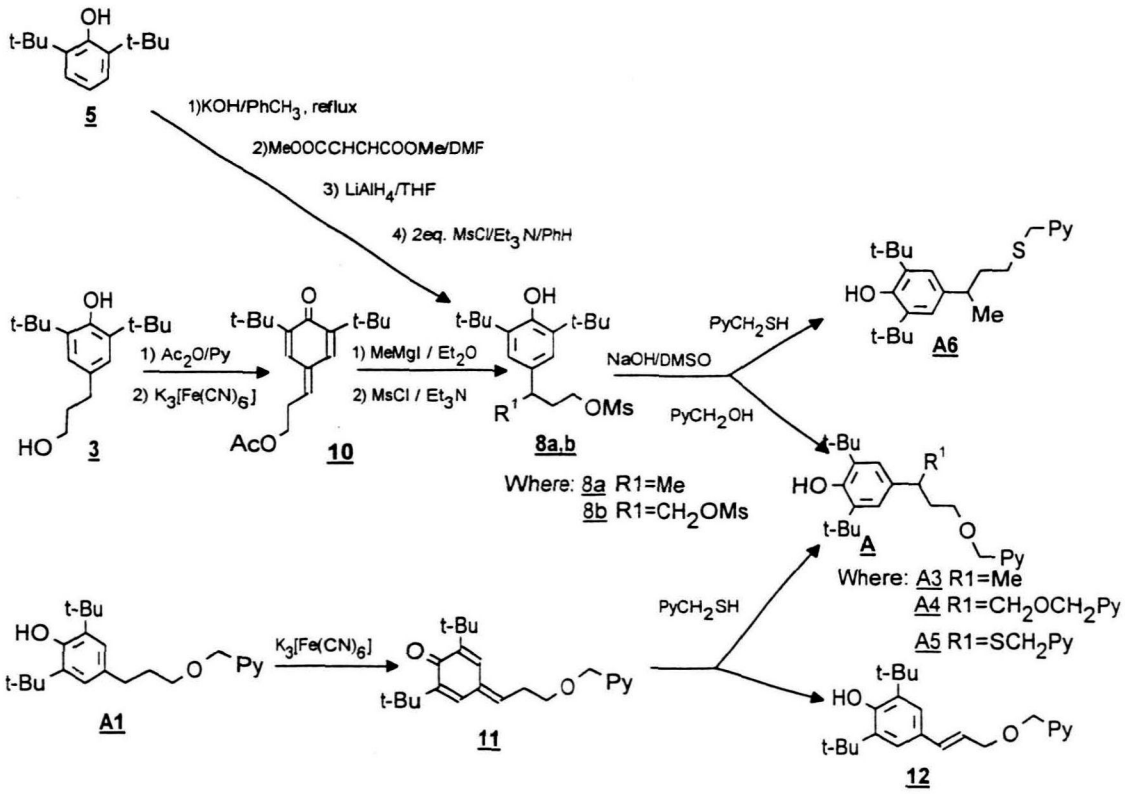


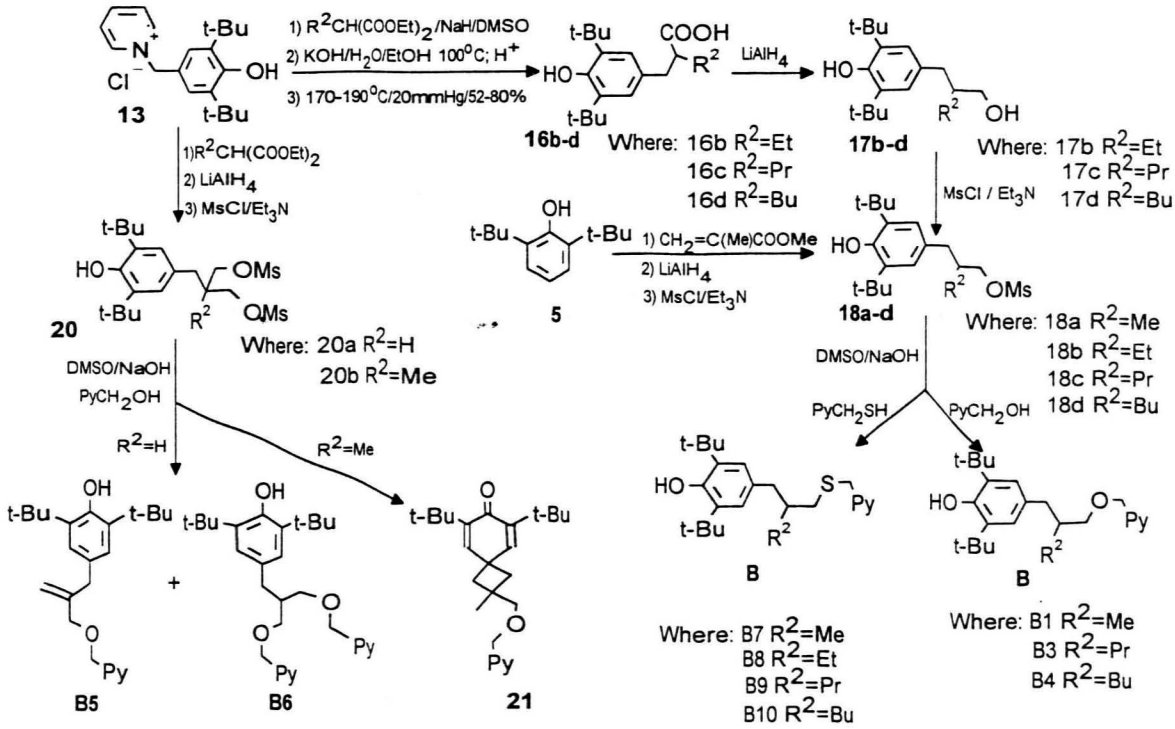
Figure 1. Structural types of Mrz 3/124 derivatives.



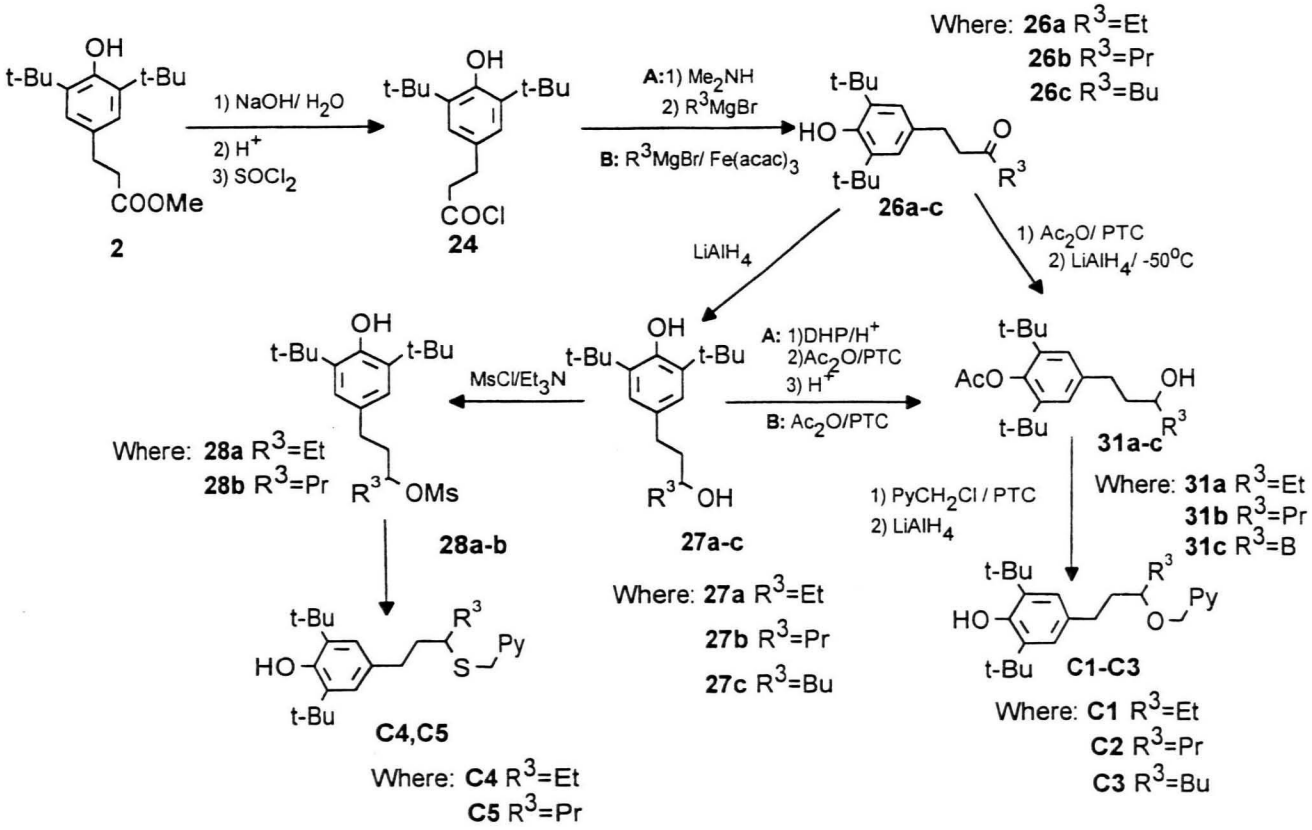
Scheme 1. Synthesis of Mrz 3/124 and corresponding thia analogue.



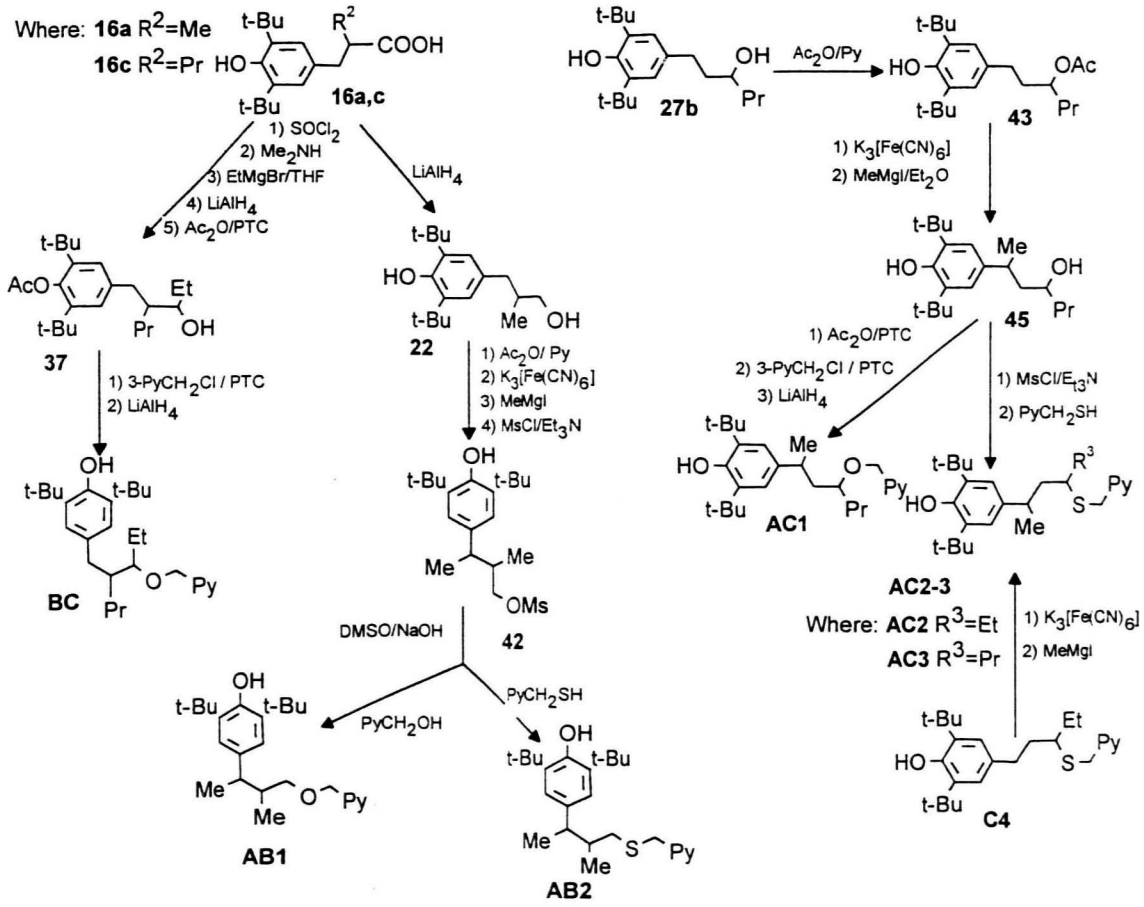
Scheme 2. Preparation of type A Mrz 3/124 derivatives



Scheme 3. Preparation of type B Mrz 3/124 derivatives



Scheme 4. Synthesis of type C Mrz 3/124 derivatives



Scheme 5. Preparation of miscelaneous type Mrz 3/124 derivatives

Supplementary material to the article

Synthesis and biological activity of 2,6-di-tert-butyl-4-[5-(3-pyridyl)-4-oxa-pentyl]phenols and their thia analogues.

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Experimental.

All ^{1}H NMR spectra were recorded on a 90MHz Bruker instrument. Melting points were detected on *Boetius* table and are uncorrected. All reactions were carried out in an argon atmosphere. Solvents and reagents used were purified according to literature (Perrin, D.D., Armarego, W.L.F., *Purification of laboratory chemicals*, 3rd ed., Pergamon Press, 1988., 391pp.) Only general procedures are presented. Yields, ^{1}H NMR data and physical constants of products are summarized in tables 1-15. The content of impurities in target compounds was less than 1% according to HPLC on 4.6×250mm *Silasorb* 600 column and 4.6×250mm *Silasorb* SPH C18 column. Mobile phases: 3-5% i-PrOH in hexanes or 20-30% dioxane in heptane, and MeCN-0.1M phosphate buffer, pH=2.5 (45:55) or MeCN-0.2M acetate buffer, pH=5.0 (70:30). Detection: UV 254nm.

Addition of a,b-unsaturated esters to 2,6-di-*tert*-butylphenol.

A

Methyl 3-(3,5-di-*tert*-butyl-4-hydroxyphenyl)-propionate (*Ionox 520, 2*).

2,6-Di-*tert*-butylphenol (5) (8.0g, 38.8 mmol) was dissolved in 7.0ml (77.7 mmol) of methyl acrylate and the solution of 900mg (8.0 mmol) of KO*t*-Bu in 20ml of *t*-BuOH was added. The reaction mixture turned green and was heated in a sealed tube at 115°C for *c.a.* 20h. The green color disappeared and pale yellow suspension was obtained. The slurry was poured into 200ml of ice-water and extracted with 3x30ml of ether. Extracts were dried, evaporated and the residue was distilled in a short-path apparatus at 0.01mbar to give 8.0g (27.4 mmol, 71%) of ester 2 as a clear oil crystallizing on standing.

B.

Diethyl 2-(3,5-di-*tert*-butyl-4-hydroxyphenyl)-succinate (6).

Potassium 2,6-di-*tert*-butylphenolate (10.0g, 41.0 mmol) was dissolved in 70ml of DMF. Diethyl maleate (21.0g, 122.1 mmol) was added and the reaction mixture was heated at 100°C for 16h. Pale yellow suspension was poured into 300ml of ice-water, neutralized with HCl to pH=6-7, and extracted with chloroform (3x70ml). Extract was dried, evaporated to dryness to give 10.3g (27.2 mmol, 66%) of diester 6 after crystallization from hexane.

Alkylation of malonic esters with 1-(3,5-di-*tert*-butyl-4-hydroxybenzyl)-pyridinium chloride.

Diethyl (3,5-di-*tert*-butyl-4-hydroxybenzyl)malonate (14a).

80% NaH in white oil (1g, 33.3 mmol) was suspended in 50ml of DMSO and dietyl malonate (5ml, 32.9 mmol) was added dropwise. After dissolving of all NaH, a solution of 3,5-di-*tert*-butyl-4-hydroxybenzyl pyridinium chloride 13 (11.0g, 33.0 mmol) in DMSO was added during 45 min at room temperature. The reaction mixture was stirred for an additional hour, poured into 1l of water and acidified to pH=6. Product 14a was extracted with EtOAc (3x150ml), extract was dried and evaporated to give pale yellow oil crystallizing on standing. Yield: 10.0g (26.5 mmol, 80%).

Hydrolysis of 3-(3,5-di-*tert*-butyl-4-hydroxyphenyl)-propionic acid esters.

3-(3,5-Di-*tert*-butyl-4-hydroxyphenyl)-propionic acid (16).

Methyl 3-(3,5-di-*tert*-butyl-4-hydroxyphenyl)-propionate (2) (150.0g, 0.51 mol) was added to a solution of NaOH (40g, 1.00 mol) in 1500 ml of water. Reaction mixture was heated at 85-90°C until clear solution was obtained. The solution was cooled to room temperature, some cloudy precipitate filtered off, and neutralized by aqueous HCl until pH=5.0. The white precipitate was filtered, washed with water (500 ml) and dried to give 135.0g (0.49 mol, 95%) of 3-(3,5-di-*tert*-butyl-4-hydroxyphenyl)-propionic acid 16.

Preparation of 3-(3,5-di-*tert*-butyl-4-hydroxyphenyl)-propionic acids.

2-Propyl-3-(3,5-di-*tert*-butyl-4-hydroxyphenyl)-propionic acid 16c.

Diester 14d (37.35g , 88.9 mmol) was dissolved in EtOH (65 ml) and a solution of KOH (24g in 25ml of H_2O) was added in one portion. The reaction mixture was refluxed for 6h. Ethanol was evaporated under reduced pressure, and 250ml of water was added under stirring. The solution was vigorously stirred and acidified with 10% HCl until pH=1. The precipitate was collected by filtration and washed with water. The amorphous solid obtained (32.0g) was placed into roud bottom flask and heated under vacuum (20 mbar) in an oil bath with stirring until decarboxylation started (*c.a.* 140°C in the bath). The reaction mixture was stirred and heated for additional 25min until the CO_2 evolution ceased, then cooled to room temperature. The crude product, containing traces of ester, was dissolved in EtOH (80ml) and the solution of KOH (10.0g in 30ml of H_2O) was added. The reaction mixture was refluxed for 3h. The solution was cooled, acidified to pH=1 with 10% HCl and product was extracted with chloroform. Extract was dried and evaporated to give crude 16c. The carboxylic acid 16c was recrystallized from hexane. Yield: 14.0g (43.7mmol, 49%).

Preparation of acyl chlorides.

3-(3,5-Di-*tert*-butyl-4-hydroxyphenyl)-propionyl chloride (24).

3-(3,5-Di-*tert*-butyl-4-hydroxyphenyl)-propionic acid (16) (50.0g, 0.18 mol) was suspended in 500 ml of dry hexane, 10ml of dry DMF were added. Freshly distilled thionyl chloride (75 ml, 1.03 mol) was added dropwise within 1h. Reaction mixture was heated under reflux for 6h, evaporated to dryness under reduced pressure at 30-35°C. The light brown residue was crystallized from hexane to give 31.4g (0.11 mol, 59%) of 3-(3,5-di-*tert*-butyl-4-hydroxyphenyl)-propionyl chloride (24). (M.p.=68-70°C)

Preparation of ketones.

A.

N,N-Dimethyl 3-(3,5-di-*tert*-butyl-4-hydroxyphenyl)-propionic acid amide (25).

94.0g (2.09 mol) of anhydrous dimethylamine was dissolved in 400ml of methylene chloride at -10°C. The solution of 3-(3,5-di-*tert*-butyl-4-hydroxyphenyl)-propionyl chloride (24) (25.7g, 0.87 mol) in 100ml of dry methylene chloride was added within 1h at -10°C. The reaction mixture was allowed to warm up to room temperature and washed with 3x100ml of 10% HCl, then with water until pH=6-7. The methylene chloride layer was

dried over Na_2SO_4 and evaporated to dryness to give 26,2g (0.86 mol, 99%) of dimethylamide 25. ^1H NMR spectrum ($CDCl_3$, TMS, δ): 1.42 (18H, s, 2*t*-Bu); 2.44-3.00 (4H, m, CH_2CH_2); 2.92 and 2.96 (6H, 2s, $N(CH_3)_2$); 5.04 (1H, s, OH); 6.98 ppm (2H, s, C_6H_2).

1-(3,5-Di-*tert*-butyl-4-hydroxyphenyl)-heptan-3-one (26c).

N,N-dimethyl 3-(3,5-di-*tert*-butyl-4-hydroxyphenyl)-propionic acid amide (25) (5.0g, 16.4 mmol) was dissolved in 50 ml of THF and added to solution of 75 mmol butylmagnesium bromide in 150 ml of THF. Reaction mixture was kept at room temperature for 170h. Usual workup gave 5.6g of light brown oil. The crude product was passed through a 20x4.5 cm *Kieselgel 60* column and eluted with hexane/ethylacetate (10/4). The product containing fraction was evaporated to give 3.8g (11.9 mmol, 73%) of ketone 26c as a pale yellow oil.

B

1-(3,5-Di-*tert*-butyl-4-hydroxyphenyl)-hexan-3-one (26b).

3-(3,5-di-*tert*-butyl-4-hydroxyphenyl)-propionyl chloride (24) (5.9g, 19,9 mmol) was dissolved in 200 ml of THF and ferric acetylacetonate (300mg, 0.85 mmol) was added. The solution was cooled to -65°C and 22.0 mmol of propylmagnesium bromide in 30 ml of THF were added within 15 min. The reaction mixture was stirred for 0.5h at -65°C and then propylmagnesium bromide (20.0 mmol) was added within 1.5h at -60- -65°C. The deep-red solution was allowed to warm up to room temperature, poured on 300g of ice, extracted with 3x150 ml of ethyl acetate. Extract was washed with brine, dried over Na_2SO_4 and evaporated to give 5.8g (19.1 mmol, 96%) of crystalline ketone 26b.

Acetylation of ketones.

1-(3,5-Di-*tert*-butyl-4-acetyloxyphenyl)-hexan-3-one (32b).

Ketone 26b (5.00g, 16.45 mmol) was dissolved in 80ml of methylene chloride and tetrabutylammonium hydrogen sulfate (1.50g, 4.25 mmol) was added. The solution was cooled to 5-7°C and diluted with 80ml of 40% NaOH solution in water. The mixture was vigorously stirred for 0.5h at 5°C and then acetic anhydride (4.5 ml, 47.69 mmol) was added dropwise within 0.5h. The stirring was continued for an additional 1h until TLC (hexane/EtOAc 10/1) showed disappearance of the starting material. The reaction mixture was diluted with 300ml of water, organic layer separated and water phase extracted with 3x75ml of CH_2Cl_2. The organic extracts were washed with water until neutral pH, dried and evaporated to give 5.03g (14.54 mmol, 88%) of acetylated ketone 32b as a clear oil.

Preparation of 3-(3,5-di-*tert*-butyl-4-hydroxyphenyl)-propan-1-ols.

A.Reduction of ketones.

1-(3,5-di-*tert*-butyl-4-hydroxyphenyl)-hexan-3-ol (27b).

Ketone 26b (10.37g, 34.0 mmol) was dissolved in 50ml of THF and added to a solution of $LiAlH_4$ (1.30g, 34.3 mmol) in 500ml of THF at -35°C within 35 min. The reaction mixture was stirred for an additional 15 min quenched with ethyl acetate. washed with saturated

tartrate solution and water, the organic layer dried over Na_2SO_4 and evaporated to give 9.66g (31.6 mmol, 93%) of alcohol 27b as a crystalline substance.

B.Reduction of esters.

3-(3,5-Di-*tert*-butyl-4-hydroxyphenyl)-2-methyl-propan-1-ol (22).

$LiAlH_4$

Ester 21a (20.0g, 65.4mmol) was dissolved in 100ml of THF and added to a solution of $LiAlH_4$ (2.5g, 65.9 mmol) in 500ml of THF. Reaction mixture was refluxed for 1h. Usual workup gave 15.3g (55.0 mmol, 83%) of alcohol 22 as a crystalline substance.

$NaBH_4$

Ester 21a (7.0g, 22.9 mmol) was dissolved in 60ml of *t*-BuOH and $NaBH_4$ (4.0g, 105.3 mmol) was added. 20ml of MeOH were added dropwise with stirring at 45-55°C within 30min. When the exotermic reaction ceased, the slurry was refluxed for 1h until TLC showed full conversion of the starting material. The reaction mixture was poured into 800ml of water, neutralized to pH=5 with HCl and filtered. The white precipitate was dried to give 5.8g (20.9 mmol, 91%) of alcohol 22.

C. Reaction of quinone methide with Grignard reagent.

2-(3,5-di-*tert*-butyl-4-hydroxyphenyl)-heptan-4-ol (45).

Crude quinomethide 44 (19.9g, 57.5 mmol) was dissolved in 150ml of THF and added to a stirred solution of 0.5 mol MeMgI in 500ml of ether. When the exotermic reaction ceased, the resulting suspension was heated at reflux for 4h. Usual workup gave 18.02g (56.31 mmol, 98%) of alcohol 45 as a clear oil.

Synthesis of mesylates.

2-(3,5-Di-*tert*-butyl-4-hydroxyphenyl)-butyl mesylate(8a).

Alcohol 7a (4.98g, 18mmol) was dissolved in 50ml of benzene. Et_3N (3.7ml, 26.5 mmol) was added and reaction mixture was cooled to 8°C. Methanesulfonyl chloride (2.0ml, 25.4 mmol) was added within 25min. The reaction mixture was stirred for an additional hour, precipitate filtered off and washed with 2x15ml of benzene. The filtrate was evaporated to dryness and treated with 15ml of cold i-propyl alcohol to give 3.37g (9.5 mmol, 53%) of mesylate 8a after crystallization from i-propyl alcohol (10ml).

Acetylation of alcohols.

1-(3,5-Di-*tert*-butyl-4-acetyloxyphenyl)-hexan-3-ol (31b).

The solution of ketone 32b (5.03g, 14.54mmol) in 20ml of THF was added to a 550mg (14.54 mmol) of $LiAlH_4$ in 150ml of THF at -60°C within 15min. The reaction mixture was stirred at -60°C for 10min, quenched at -60°C with 15ml of MeOH and washed with tartrate solution. The organic layer was dried over Na_2SO_4 and evaporated to give 4.87g (14.00 mmol, 96%) of alcohol 27b as a clear oil.

1-(3,5-Di-*tert*-butyl-4-acetyloxyphenyl)-hexan-3-ol (31b).

Tetrabutylammonium sulfate (250mg, 0.74 mmol) was added to a solution of 1.00g (3.27 mmol) 1-(3,5-di-*tert*-butyl-4-hydroxyphenyl)-hexan-3-ol (27b) in 15ml of methylene chloride. The mixture was cooled to +5°C, diluted with 15ml of 40% NaOH and stirred for

0.5h at +5°C. 350μl (3.71 mmol) of acetic anhydride were added in one portion and stirring continued for 2h. Usual workup gave 1.12g of a pale yellow oil, which was chromatographed on a 30x2.4 cm *Kieselgel 60* column with hexane/ethylacetate (10/1) to give 0.81g (2.33 mmol, 71%) of acetylated phenol 31b as a clear oil.

Preparation of pyridyl ethers.

A.*via mesylates.*

2-(3,5-Di-*tert*-butyl-4-hydroxyphenyl)-6-(3-pyridyl)-5-oxahexane (A3).

Picolyl alcohol (2.2g, 20 mmol) was mixed with 1.60g (40 mmol) of fine powdered NaOH and 10ml of DMSO. The mixture was heated with stirring at 120°C for 1h, then cooled to 15°C and solution of mesylate 8a (3.37g, 9.5 mmol) in 7ml of DMSO was added during 2h. The reaction mixture was stirred overnight, poured into 450ml of water to give pale yellow emulsion, crystallizing on standing. Filtration yielded 2.2g (6.0 mmol, 63%) of crude ether A3, which was purified by crystallization from hexane.

In case of *bis*-ether B6 reaction mixture after addition of mesylate was heated at 100°C for 2h.

B *via PTC.*

1-(3-Pyridyl)-3-[2-(3,5-di-*tert*-butyl-4-acetyloxy-phenyl)-ethyl]-2-oxahexane (33b).

Tetrabutylammonium hydrogen sulfate (1.70g, 5.00mmol) and picolyl chloride hydrochloride (2.00g, 12.19mmol) were added to a solution of alcohol 31b (1.71g, 4.91mmol) in toluene (10ml). The resulting slurry was degassed, saturated with argon and cooled to 0°C. 10ml of 50% NaOH were added and the reaction mixture was vigorously stirred for 9h. Dark brown reaction mixture was diluted with 50ml of water and 50ml of ether. Organic layer was separated, the aqueous phase was washed with ether (4x25ml). Extract was washed with water until pH=6-7, dried and evaporated to give 4.16g of brown oil. Crude reaction mixture was separated on a 40x24cm *Kieselgel 60* column (hexane/ ethylacetate 10/4) to give 920mg (2.10 mmol, 42%) of ether 33b as a clear oil.

Deprotection of ethers.

1-(3-Pyridyl)-3-[2-(3,5-di-*tert*-butyl-4-hydroxy-phenyl)-ethyl]-2-oxahexane (C2).

Acetylated ether 33b (920mg, 2.10 mmol) was dissolved in 5ml of THF and added to a solution of $LiAlH_4$ (80mg, 2.11mmol) in 40ml of THF. The reaction mixture was heated at reflux for 2h. Usual workup gave 660mg (1.66 mmol, 79%) of deprotected ether C2 as white crystalline substance.

Preparation of thio-ethers.

2,6-Di-*tert*-butyl-4-[5-(3-pyridyl)-4-thiapentyl]-phenol (A2).

S-(3-Picolyl)isothiourea dihydrochloride (4.8g, 20mmol) was dissolved in 20ml of DMSO and cooled to 10°C. Powdered NaOH (3.2g, 80mmol) was added in one portion and resulting mixture was stirred for 10 min at 10-15°C. A solution of mesylate 4 (6.84g, 20mmol) in 20ml of DMSO was added within 10 min at 15-20°C. The reaction mixture was stirred at room temperature for 1h and poured into ice-water. Oily solid precipitated was

extracted with chloroform, extract washed with water and dried. Resulting oil crystallized by treatment with hexane to give 5.56g (15mmol, 75%) of ether A2.

In case, if no crystalline substances were obtained by treatment with hexane, chromatography on *Kieselgel 60* was used to isolate pure products (eluent: hexane/EtOAc 10/4).

Acetylation of alcohols.

1-(3,5-di-*tert*-butyl-4-hydroxyphenyl)-hex-3-yl acetate (43).

Alcohol 27b (32.31g, 0.106 mol) was dissolved in 150ml of pyridine, and acetic anhydride (15ml, 0.159 mol) was added at room temperature. Reaction mixture was stirred for 0.5h and poured into water (750ml). Product was extracted with EtOAc (3x150ml), extract was dried and evaporated to give 33.06g (95mmol, 90%) of acetylated product 43.

Syntheses of quinone methides.

2,6-di-*tert*-butyl-4-(3-acetyloxyhex-1-ylidene)-cyclohexa-2,5-dien-1-one (44).

20.0g (57.5 mmol) of acetylated alcohol 43 in 500ml of benzene were added at 7-10°C to a vigorously stirred degassed solution of $K_3[Fe(CN)_6]$ (76.0g) and NaOH (60g) in 500ml of water. Reaction mixture turned emerald green. Stirring was continued for 2.5h at 10°C until the green color disappeared. The organic layer was separated, washed with water until pH=7, dried over Na_2SO_4 and evaporated at 30°C to give 19.9g (57.5 mmol) of quinomethide 44 as a bright yellow oil.

1-(3,5-di-*tert*-butyl-4-hydroxybenzyl)-pyridinium chloride (13).

Pyridine hydrochloride (1.46g, 12.6 mmol) was dissolved in 25 ml of MeCN and Ionox-100 (3.00g, 12.7 mmol) was added in one portion. Reaction mixture was refluxed under argon 5h. White precipitate 13 was filtered off, washed with dry MeCN (20 ml) and ether (15 ml) and dried *in vacuo*. Yield: 3.50g (10.4 mmol, 83%). ^{1}H NMR spectrum (DMSO-d_6, TMS, δ): 1.37 (18H, s, 2*t*-Bu); 5.74 (2H, s, CH_2N^+); 7.24 (1H, bs, OH); 7.31 (2H, s, C_6H_2); 8.15 (1H, m, Py-5H); 8.58 (1H, m, Py-4H); 9.21ppm (2H, m, Py-2H&Py-6H).

2-Methyl-2-(3-pyridylmethyloxymethyl)-6,8-di-*tert*-butylspiro-[3,5]-nona-5,8-diene-7-one (21).

Synthesis was carried out according to method for the preparation of B5 and B6. Reaction mixture was poured into ice-water, extracted with chloroform (3x100ml), extracts were dried, evaporated to dryness and resulting oil chromatographed on *Kieselgel 60*. Melting point: 92-93°C, yield: 47%. ^{1}H NMR spectrum ($CDCl_3$, TMS, δ): 1.14 (9H, s, *t*-Bu); 1.22 (9H, s, *t*-Bu); 1.30 (3H, s, CH_3); 1.96 (2H, d, J=12.0Hz, CCH_2C); 2.43 (2H, d, J=12.0Hz, CCH_2C); 3.32 (2H, s, CH_2O); 4.61(2H, s, CH_2Py); 6.82 (2H, s, C_6H_2); 7.27 (1H, m, Py-5H); 7.69 (1H, m, Py-4H); 8.56ppm (2H, m, Py-2H&6H).

2,6-Di-*tert*-butyl-4-[1-(3-pyridylmethylthio)-3-(3-pyridylmethyloxy)-propyl]phenol (A5)

S-(3-Picolyl)isothiourea dihydrochloride (2.4g, 10mmol) was dissolved in 15ml of DMSO and cooled to 6°C. Powdered NaOH (1.6g, 40mmol) was added in one portion and

resulting mixture was stirred for 5 min at 8-10°C. A solution of quinone methide 11 (3.54g, 10mmol) in 10ml of DMSO was added dropwise within 10 min at 10°C. Reaction mixture was stirred for an additional hour at room temperature and poured into ice-water. Oily precipitate was extracted with chloroform, extract washed with water, dried and evaporated to give pale yellow oil. Chromatography on *Kieselgel 60* (hexane/EtOAc) afforded 2.8g (5.9mmol, 59%) of ether A5. Product was crystallized from hexane/i-PrOH to give pure substance.

Evaluation of lipid-lowering properties.

The experiment was carried out in groups of male SD-rats (n=5 rats per measurement).

Test compounds were suspended in Cremophor (10% in distilled water). Dissolution of the preparation was achieved on a magnetic stirrer, where the solutions had to be placed up to the administration.

Single dose (0.2 mmol/kg) of the test substance or the vehicle only were administered to fasting (24h) normolipidemic rats by the *per oral* route. 240 or 360 minutes after application blood samples were taken and the concentration of serum-triglycerides was estimated by a standard method (GPO-PAP method, E. Merck, Darmstadt, Germany). Nicotinic acid served as standard. Data are given as lowering % *vs* vehicle control.

Evaluation of anti-oxidative properties.

The anti-oxidative properties of the test compounds were evaluated by two *in vitro* assays: the determination of the lag phase for the formation of conjugated dienes according to Esterbauer et al. and the determination of thiobarbituric acid reactive substances (TBARS) according to the method of Yagi et al.

Lag time of conjugated dienes formation.

Low-density lipoproteins (LDL, d=1.019-1.063) were isolated by ultracentrifugation from human plasma sampled in EDTA-coated vials.

Protein was determined by the Lowry method using bovine serum albumin as a standard. Test substances were dissolved in ethanol.

For assaying the lag time 10µl of the drug solution, 10µl Cu^{2+} and phosphate buffered saline (PBS) were added to 200µg of LDL to give 1ml test solution with final concentrations of 10µM Cu^{2+}, 0.5% ethanol and 5 or 20µM of the test substance. Controls received ethanol only. Air was bubbled through the solution. The dosage of estimation at 234min. Induction of the formation of conjugated dienes was recorded by spectrophotometer.

The lag phase till formation of conjugated dienes is calculated from the graphs. NDGA was used as reference compound. Experiments were done in duplicates.

Data are given as % *vs* vehicle control.

TBARS formation.

LDL was prepared and protein was determined as above. Test substances were dissolved as above.

For the assay 200μg of LDL was incubated in 1ml HAM F 10 medium for 24h at 37°C in the presence of 10μM Cu^{2+} with cr without the preparation (10 or 20μM). Controls received ethanol only.

Total TBARS were determined by adding of 0.5ml aliquot to 0.75ml of 0.67% TBA in 0.05M NaOH +0.75ml of 20% trichloroacetic acid. After heating in a water bath (90°C) for 60 min the samples were cooled to room temperature and centrifuged at 5000 rpm (Varifuge, Hereaus, Germany) for 10min. Optical density was read at 532nm. NDGA was used as a reference substance, experiments were done in triplicates.

Data are given as % inhibition *vs* vehicle control.

Evaluation of anti-proliferative properties.

Smooth muscle cell (SMC) proliferation was determined using SMC from Wistar-rat aorta.

The aortas were dissected, the adventitia removed and the intimal side was gently scraped with a scalpel to remove all endothelium. Explants 1x1mm were prepared and placed with the luminal side down in tissue culture multiwells.

The explants were attached to the surface but before they started to dry out they were supplemented with Dulbecco's Minimal Essential Medium (DMEM) + 0.5% fetal calf serum (FCS).

For assaying the anti-proliferative activity of test substances subcultured SMC were used.

10'000 cells per well were suspended in DMEM+ 10% FCS. After having adhered for 4h, test compounds (50μM in 0.5% DMSO, dissolution in pure DMSO and ultrasonification for 10min) were added. Control cells received 0.5% DMSO only. After 3 days cells were counted using a Coulter cell counter (Coulter Electronics, Germany) and the reduction in cell proliferation was quantified. L44-0 was used as reference compound. 8 experiments per compound were done.

Data are given as inhibition % *vs* vehicle control.

Table 1. Preparation of pyridyl ethers.

# of cmpd.	R	Method of prepn.	M.P., (°C)	Yield, (%)
A1	R^1=R^2=R^3=H	A	104-5	76
A2	R^1=Me; R^2=R^3=H	A		53
A3	R^1=CH_2OCH_2Py; R^2=R^3=H	A		23
A4	R^1=SCH_2Py; R^2=R^3=H	C	95-6	59
B1	R^2=Me; R^1=R^3=H	A		47
B3	R^2=Pr; R^1=R^3=H	A	99-101	62
B4	R^2=Bu; R^1=R^3=H	A	71-2	43
B5	R^2=CH_2=; R^1=R^3=H	A (separated from B-6)	95-6	37 (Total conv.: 56%)
B6	R^2= CH_2OCH_2Py; R^1=R^3=H	A (separated from B-5)	75-6	19 (Total conv.: 56%)
C1	R^3=Et; R^1=R^3=H	B	82-3	Condensation: 41 Reduction: 95 Total: 39
C2	R^3=Pr; R^1=R^3=H	B	63-4	Condensation: 41 Reduction: 96 Total: 39
C3	R^3=Bu; R^1=R^3=H	B	oil	Condensation: 38 Reduction: 87 Total: 33
AC1	R^1=Me; R^2=H; R^3=Pr	B	oil	Condensation: 36 Reduction: 91 Total: 33
BC	R^1=H; R^2=Pr; R^3=Et	B	oil *(mixture of diastereomers 1:1)*	Condensation: 55 Reduction: 99 Total: 54
AB1	R^1=Me; R^2=Pr; R^3=H	A	56-7	39

Method A: reaction of mesylate with pyridylcarbinol.
Method B: alkylation of protected alcohol with picolyl chloride and deprotection *via* $LiAlH_4$.
Method C: reaction of quinone methide with nucleophile.

Table 2. ^{1}NMR spectra of 2,6-di-tert-butyl-4-[5-(3-pyridyl)-4-oxapentyl]phenols.

# of cmpd.	R^1, R^2, R^3	δ, TMS, ppm								
		t-Bu	ArCCH	ArCH	CHO	CH_2Py	ArOH	Ar	Py	R^1, R^2, R^3
A1	$R^1=R^2=R^3=H$	1.38	1.56-2.16 (2H,m)	2.60 (2H,m)	3.49 (2H, t, J=6.0Hz)	4.49 (2H,s)	5.00 (1H,s)	6.93 (2H, s)	7.22 (1H, m,Py5H) 7.64 (1H, m,Py4H) 8.49 (2H, m,Py2&6H)	-
A3	R^1=Me; $R^2=R^3=H$	1.42	1.55-2.18 (2H,m)	2.80 (1H,m)	3.40 (2H, m)	4.44 (2H,s)	5.00 (1H,s)	6.93 (2H, s)	7.20 (1H, m,Py5H) 7.58 (1H, m,Py4H) 8.38-8.56 (2H,m, Py2H&6H)	R^1: 1.23 (3H, d,J=6.8Hz)
A5	$R^1=SCH_2Py$; $R^2=R^3=H$	1.41	2.20 (2H, m)	3.78 (1H,t,J=7.5 Hz)	3.60 (2H, m)	4.41 (2H, s)	5.20 (1H, s)	7.00 (2H, s)	7.05-7.30 (1H, m, Py5H); 7.51 (1H, m, Py4H); 8.42 (2H, m, Py2H&6H).	R^1: 3.45 (2H, s); 7.05-7.30 (1H, m, Py5H); 7.51 (1H, m, Py4H); 8.42 (2H, m, Py2H&6H).
A4	$R^1=CH_2O$-CH_2Py; $R^2=R^3=H$	1.40	1.71-2.38 (2H, m)	2.62-3.16 (1H, m)	3.24-3.84 (2H, m)	4.24. (2H, s)	5.04 (1H, s)	6.96 (2H, s)	7.07-7.31 (1H,m,Py5H) 7.44-7.69 (1H,mPy4H) 8.49 (2H, m, Py2H&6H)	R^1: 3.24-3.84 (2H, CH_2O) ; 4.47 (2H, CH_2Py); 7.07-7.31; 7.44- 7.69; 8.49 PyH.
B1	R^2=Me; $R^1=R^3=H$	1.42	1.86-2.29 (1H,m)	2.29-2.89 (2H, m)	3.38 (2H, m)	4.51 (2H, s)	5.00 (1H, s)	6.91 (2H, s)	7.27 (1H, m, Py5H) 7.67 (1H, m, Py4H) 8.44-8.62 (2H,m, Py2H&6H)	R^2: 0.96 (3H, d,J=6.0Hz)
B3	R^2=Pr; $R^1=R^3=H$	1.42	1.80 (1H, m)	2.56 (2H, d, J=7.0Hz)	3.32 (2H, d, J=5.5Hz)	4.46 (2H, s)	5.00 (1H, s)	6.91 (2H, s)	7.24 (1H, m, Py5H); 7,61 (1H, m, Py4H); 8.51 (2H, m, Py2H&6H)	R^2: 0.88 (3H, m); 1.15-1.45 (4H, m)
B4	R^2=Bu; $R^1=R^3=H$	1.42	1.85 (1H, m)	2.56 (2H, m)	3.32 (2H, m)	4.46 (2H, s)	5.00 (1H, s)	6.90 (2H, s)	7.22 (1H, m, Py5H) 7.61 (1H, m, Py4H) 8.49 (2H, m, Py2H&6H)	0.86 (3H, t, J=6.5Hz) 1.10-1.40 (6H, m)
B6	R^2= CH_2OCH_2Py; $R^1=R^3=H$	1.40	2.15 (1H, m)	2.64 (2H, d, J=8.0Hz)	3.48 (2H, d, J=5.0Hz)	4.48 (2H, s)	5.03 (1H, s)	6.92 (2H, s)	7.21 (1H, dd, J_1=4.5Hz, J_2=8.0Hz) 7.59 (1H, dt, J_1=8.0Hz, J_2=2.0Hz); 8.50 (2H, m, Py2H&6H)	R^2: 3.48 (2H, d, J=5.0Hz); 4.48 (2H, s); 7.21 (1H, dd, J_1=4.5Hz, J_2=8.0Hz) 7.60 (1H, dt, J_1=8.0Hz, J_2=2.0Hz); 8.50 (2H, m, Py2H&6H)
B5	R^2= CH_2=; $R^1=R^3=H$	1.40	-	3.31 (2H, s)	3.94 (2H, s)	4.48 (2H, s)	5.07 (1H, s)	6.96 (2H, s)	7.21 (1H, dd, J_1=4.5Hz, J_2=8.0Hz) 7.60 (1H, dt, J_1=8.0Hz, J_2=2.0Hz); 8.50 (2H, m, Py2H&6H)	R^2: 4.96 and 5.11 (2H, 2d, J=2Hz)
C1	R^3=Et; $R^1=R^2=H$	1.42	1.67-1.98 (2H, m)	2.49-2.78 (2H, m)	3.40 (1H, m)	4.53 (2H, s)	5.02 (1H, s)	6.98 (2H, s)	7.16-7.36 (1H, m, Py5H) 7.69 (1H, m, Py4H) 8.53 (2H, m, Py2H&6H)	0.93 (3H, t, J=7.2Hz) 1.47-1.73 (2H, q, J=7.2Hz)
C2	R^3=Pr; $R^1=R^2=H$	1.42	1.67-2.00 (2H, m)	2.44-2.56 (2H, m)	3.27-3.60 (1H, m)	4.51(2H, s)	5.03 (1H, s)	6.96 (2H, s)	7.11-7.36 (1H, m,Py5H) 7.56-7.76 (1H, m,Py4H) 8.44-8.62 (2H, m, Py2H&6H)	0.91 (3H, m) 1.18-1.76 (4H, m)
C3	R^3=Bu; $R^1=R^2=H$	1.41	1.74-2.04 (2H, m)	2.49-2.82 (2H, m)	3.44 (1H, m)	4.53 (2H, s)	5.04 (1H, s)	6.96 (2H, s)	7.11-7.36 (1H, m,Py5H) 7.69 (1H, m, Py4H) 8.56 (2H, m, Py2H&6H)	0.91 (3H, m) 1.02-1.74 (6H, m)
AC1	R^1=Me; R^2=H R^3=Pr	1.40	1.53-2.04 (2H, m)	2.78 (1H, m)	2.98-3.36 (1H, m)	4.36 (2H, m)	5.02 and 5.04 (1H, 2s)	6.90 and 6.93 (2H, 2s)	7.02-7.31 (1H, m,Py5H) 7.31-7.69 (1H, m,Py4H) 8.42 (2H, m, Py2H&6H)	R^1: 1.16-1.30 (3H, m); R^3: 0.69-1.00 (3H, m) 1.00-1.53 (4H, m)
BC	R^1=H; R^2=Pr R^3=Et	1.40	1.69-2.22 (1H, m)	2.40-2.87 (2H, m)	3.00-3.30 (1H, m)	4.33-4.35 (2H, m)	5.00 and 5.04 (1H, 2s)	6.91 (2H, s)	7.16-7.36 (1H, m,Py5H) 7.44-7.77 (1H, m,Py4H) 8.42-8.62 (2H,m,Py2H&6H)	R^2: 0.71-1.00 (3H, m) 1.00-1.69 (4H, m); R^3: 0.71-1.00 (3H, m) 1.00-1.69 (2H, m)
AB1	R^1=Me; R^2=Me R^3=H	1.41	1.60-2.10 (1H, m)	2.50-2.95 (1H, m)	3.00-3.45 (2H, m)	4.39 and 4.49 (2H, 2s)	5.00 (1H, s)	6.89 (2H, s)	7.22 (1H, m, Py5H) 7.61 (1H, m, Py4H) 8.51 (2H, m, Py2H&6H)	R^1: 1.21-1.24 (3H, m); R^2: 0.78-0.97 (3H, m)

Table 3. Preparation and ^1H NMR spectra 2,6-di-*tert*-butyl-4-[5-(3-pyridyl)-4-thia pentyl]phenols.

# of cmpd.	R^1, R^2, R^3	δ, TMS, ppm									M. P., °C	Yield, %
		t-Bu	R^1, R^2, R^3	ArCCH	ArCH	ArOH	CHS	CH_2Py	Ar	Py		
A2	R^1 = R^2=R^3=H	1.42	-	1.87 (2H, m)	2.59 (2H, m)	5.04 (1H, s)	2.45 (2H, m)	3.67 (2H, s)	6.93 (2H, s)	7.19 (1H, m, Py5H); 7.60 (1H, m, Py4H); 8.47 (2H, m, Py2H&6H)	59-60	75
A6	R^1=Me; R^2=R^3=H	1.42	R^1: 1.20 (3H, d,J=7.0Hz)	1.78 (2H, m)	2.72 (1H, m)	5.04 (1H, s)	2.32 (2H, m)	3.63 (2H, s)	6.92 (2H, s)	7.16 (1H, m, Py5H) 7.53 (1H, m, Py4H) 8.44 (2H, m,Py2H&6H)	105-6	70
B7	R^2=Me; R^1=R^3=H	1.42	R^2: 0.93 (3H, d, J=6.0Hz)	1.90 (1H, m)	2.53 (2H, d, J=6.0Hz)	5.04 (1H, s)	2.38 (2H, d, J=6.0Hz)	3.63 (2H, s)	6.89 (2H, s)	7.18 (1H, m, Py5H) 7.51 (1H, m, Py4H) 8.44 (2H, m, Py2H&6H)	63-4	63
B8	R^2=Et; R^1=R^3=H	1.41	R^2: 0.87 (3H, m), 1.20-1.50 (2H,m)	1.72	2.54 (2H, d, J=7.0Hz)	5.05 (1H, s)	2.36 (2H, d, J=6.0Hz)	3.59 (2H, s)	6.90 (2H, s)	7.15 (1H, m, Py5H); 7.28 (1H, m, Py4H); 8.42 (2H, m, Py2H&6H)	oil	54
B9	R^2=Pr; R^1=R^3=H	1.41	R^2: 0.87 (3H, m); 1.15-1.45 (4H,m)	1.78 (1H, m)	2.54 (2H, dd, J_1=1.7Hz, J_2=6.5Hz)	5.03 (1H, s)	2.35 (2H, dd, J_1=1.2Hz, J_2=6.0Hz	3.59 (2H, s)	6.90 (2H, s)	7.15 (1H, m, Py5H); 7.49 (1H, m, Py4H); 8.43 (2H, m, Py2H&6H)	82-3	48
B10	R^2=Bu; R^1=R^3=H	1.41	R^2: 0.86 (3H, m); 1.10-1.40 (6H, m)	1.72 (1H, m)	2.54 (2H, d, J=6.5Hz)	5.02 (1H, s)	2.36 (2H, dd, J_1=1.0 Hz, J_2=6.0Hz	3.59 (2H, s)	6.90 (2H, s)	7.15 (1H, m, Py5H); 7.49 (1H, m, Py4H); 8.43 (2H, m, Py2H&6H)	80-1	32
C4	R^3=Et; R^1=R^2=H	1.42	R^3: 0.94 (3H, t, J=7.0Hz) 1.50-1.95 (2H, m)	1.50-1.95 (2H, m)	2.30-2.75 (2H, m)	5.03 (1H, s)	2.30-2.75 (1H, m)	3.66 (2H, s)	6.93 (2H, s)	7.18 (1H, m, Py5H0 7.60 (1H, m, Py4H) 8.44 (2H, m, Py2H&6H)	73-4	53
C5	R^3=Pr; R^1=R^2=H	1.42	R^3: 0.83 (3H, m); 1.20-1.45 (4H, m)	1.60-1.95 (2H, m)	2.30-2.75 (2H, m)	5.04 (1H, s)	2.30-2.75 (2H, m)	3.66 (2H, s)	6.93 (2H, s)	7.18 (1H, m, Py5H); 7.60 (1H, m, Py4H); 8.44 (2H, m, Py2H&6H)	75-6	45
AC3	R^3=Pr; R^1=Me R^2=H	1.42	R^1: 1.17 (3H, d, J=7.0Hz); R^3: 0.71-0.77 (3H, m); 1.25-1.9 (4H, m)	1.25-1.90 (2H, m)	2.70-3.10 (1H, m)	5.03 and 5.06 (1H, 2s)	2.05-2.45 (1H, m)	3.59 (2H, s)	6.95 (2H, s)	7.05-7.25 (1H, m,Py5H); 7.30-7.55 (1H, m,Py4H); 8.40 (2H, m, Py2H&6H).	78-9	63
AB2	R^3=H; R^1=Me R^2=Me	1.40	R^1: 1.16 (3H, d,J=7.5Hz); R^2: 0.84-1.00 (3H, m)	1.55-1.95 (1H, m)	2.10-2.85 (1H, m)	5.13 and 5.17 (1H, 2s)	2.10-2.85 (2H, m)	3.50 and 3.60 (2H, 2s)	6.88 and 6.91 (2H, 2s)	7.10 (1H, m, Py5H) 7.35-7.51(1H,m,Py4H) 8.40 (2H, m, Py2H&6H)	oil	50
AC2	R^2=H; R^1=Me R^3=Et	1.41	R^1: 1.16-1.18 (3H, m); R^3: 0.82-0.90 (3H, m); 1.45-1.85 (2H, m)	1.45-1.85 (2H, m)	2.70-3.10 (1H, m)	5.05 (1H, bs)	2.05-2.40 (1H, m)	3.55 (2H, s)	6.93 (2H, s)	7.05-7.25 (1H, m,Py5H); 7.30-7.60 (1H, m,Py4H); 8.40 (2H, m, Py2H&6H)	oil	68

2. Pielikums

The chemical synthesis of presumptive 2,6-di-*tert*-butyl-4-[5-(3-pyridyl)-4-oxapentyl]phenol (*Nicanartine, Mrz 3/124*) metabolites.

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Summary. Eight potential metabolites of 2,6-di-*tert*-butyl-4-[5-(3-pyridyl)-4-oxapentyl]phenol, an hypolipidemic and antiatherosclerotic preparation, were synthesized. Oxidative metabolism of both 2,6-di-*tert*-butyl-4-methylphenol moiety and pyridyl-3-carbinol residue was simulated. Reactions of 2,6-di-*tert*-butyl-4-[5-(3-pyridyl)-4-oxapent-1-ylidene]cyclohexa-2,5-dien-1-one, as primary metabolite, with glutathione and cysteine were investigated and water soluble adducts were obtained under phase transfer conditions.

Keywords. Butylated hydroxytoluene / Pyridyl-3-carbinol / Metabolism / Quinone methide

Introduction.

Since 1954 the 2,6-di-*tert*-butyl-4-methylphenol (1) (BHT, butylated hydroxy toluene) has been one of the most widely used preservatives added to foods, cosmetics and drugs. Combination of the BHT residue with a nicotinic acid precursor - pyridyl-3-carbinol[1] gave compound Mrz 3/124 (*Nicanartine*) (2) with high hypolipidemic and antiatherosclerotic activity.

In this paper we wish to report the chemical synthesis of several potential metabolites of Mrz 3/124. The 2,6-di-*tert*-butylphenol moiety suggested us to look for analogous products of BHT (1) itself. The oxidative metabolism of butylated hydroxy toluene (1) is well studied and the initial metabolite - quinone methide 3 and subsequent products 4-7 are well known [2,3,4]. On the other hand structures 8 - 11 are analogues of known compounds 4 - 6 (figure 2). If the carbon chain of the Mrz 3/124 undergoes metabolic degradation, quinone 7 could be produced both from pyridyl ether 2 and BHT (1). This means, that quinone 7 is a general metabolite for all BHT derived compounds and requires no analogues in our scheme.

Glutathione (GSH) and cysteine (Cys) possibly are molecules capable to form conjugates 12 and 13 respectively with quinone methide 3, protecting thus organism from toxicity of 3[5,6,7]. More recently the capability of quinone methide 3 to form adducts 12 with glutathione was demonstrated[8] *in vitro*. So the corresponding Mrz 3/124 derived analogues 14 and 15 also are objects of interest in the metabolism research of pyridyl ether 2 (figure 3):

Finally the oxidative metabolism of pyridylcarbinol moiety was represented by the synthesis of N-oxide 16 of ether 2 and corresponding rearrangement products 17 and 18[9] (figure 4).

So the aim of our research was investigations of the chemical synthesis of oxidation products 8 - 11 and both N-oxide 16 and rearrangement products 17 and 18 (figure 4) as well as the examination of the quinone methide 8 reactions with glutathione and cysteine to obtain adducts 14 and 15 (figure 4).

Chemistry.

Treatment of pyridyl ether 2 with *m*-chloroperbenzoic acid (MCPBA) gave N-oxide 16. This was rearranged to pyridones 17 and 18 according to literature[10], however we preferred trifluoroacetic anhydride in methylene chloride to acetic anhydride as a reagent. This allowed us to perform the transformation at -70°C thus avoiding the acylation of the phenolic OH group. Both isomers were separated by LC on *Kieselgel 60*.

Attempting to synthesize oxidation products 8 - 11 we tried to oxidize 2 with both benzyltrimethylammonium tribromide[11] and methyl-3-(1,1,1-trimethylhydrazinium)-propionate tribromide[12]. Treatment of 2 with 1eq. of oxidizing agent did not afford the desired product 10, instead of this quinol 9 was obtained. Two equivalents of both reagents gave ketone 11 in good yield. Reduction of 11 with $LiAlH_4$ under mild conditions afforded alcohol 10, whereas treatment with $NaBH_4$ in iso-propyl alcohol gave phenol 2 in nearly quantitative yield.

Oxidation of Mrz 3/124 (2) with potassium ferricyanide in aqueous NaOH/PhH system[13] (figure 4) gave quinone methide 8 as a bright yellow oil. Attempts to purify 8 *via* flash chromatography failed due to rapid isomerization to a styrene

derivative 19. Transformation from quinone methides to styrenes is already described in literature[14]. Isomerization of quinoid compound 8 to the unsaturated product 19 was favored not only by silica gel, but occurred also by standing at room temperature or treatment of the quinone methide with various nucleophiles under basic conditions. The problem was solved by the use of phase transfer conditions (PTC) (figure 5):

Quinone methide 8 was treated with glutathione (GSH), cysteine (Cys) in methylene chloride / water system under neutral conditions in the presence of tetrabutylammonium hydrogen sulfate. Addition products with GSH 14 and Cys 15 were obtained as water soluble substances.

Biology.

Toxicological studies of Nicanartine both on rats and dogs were carried out.

Urine, liver bile and gallbladder bile derived from a toxicological study in dogs have been extracted with ether and subjected to a suitable GC-MS evaluation. Identification of metabolites was acchieved *via* chromatographic comparison with reference standards synthesized or masspectra of known BHT metabolites.

Following compounds have been identified:

Mrz 3/292, 297, 301, 303, 304, 305, 323, 124, 2,6-di-*tert*-butyl-p-benzoquinone (7, further DBPB), 2,6-di-*tert*-butyl-4-methylene-2,5-cyclohexadienone (3).

These results have been confirmed by LC-MS and LC-MS/MS methods. Additionally compounds Mrz 3/293, 296 and 178 were found. Metabolite Mrz 3/298 was detected in urine only.

Moreover gluthathion adducts of Nicanartine have been detected

Biological samples derived from a toxicological study on rats have been treated with ether and subjected to GC-MS analysis only. Following compounds were found;

Liver bile:

Mrz 3/292, 296, 297, 301,303, 304, 305, DBPB (7), 2,6-di-*tert*-butyl-4-(3-hydroxypropyl)-4-hydroxy-2,5-cyclohexadienone (see Fig.6, 19) and 2,6-di-tert-butyl-p-hydroquinone (see Fig.6, 20, further DBHC)

Urine:

traces of Nicanartine only

Serum:

Mrz 301,304

Organs (such as liver, stomach, brain, lung, heart, kidneys, adipose tissue):

Mrz 3/223, 292, 295, 301, 303, 304, DBPB (7), DBHC(20).

Results.

We have succeeded chemical syhthesis of presumptive metabolites of 2,6-di-*tert*-butyl-4-[5-(3-pyridyl)-4-oxapentyl]phenol directly from the parent compound. Different oxidazing agents selectively gave products corresponding to known BHT analogues. Substances obtained are stable enough to be used as standards in investigation of the metabolism of pyridyl ether 2. Moreover, quinone methide 8 turned out to be highly attractive starting material for convenient synthesis of functionalized *Nicanartine* derivatives. Adducts with glutathione and cysteine were obtained in one step procedure under phase transfer conditions.

Analysis of biological materials derived from toxicological studies on both dogs and rats revealed presence of both synthesized metabolites of *Nicanartine* and from BHT derived products. Certain diversity of metabolic degradation of *Nicanartine* in various organs is observed.

Experimental.

All ^1H NMR spectra were recorded on a 90MHz Bruker instrument. Melting points were detected on *Boetius* table and are uncorrected. All reactions were carried out in an argon atmosphere. Solvents and reagents used were purified according to literature[15]. 2,6-Di-*tert*-butyl-4-[5-(3-pyridyl)-4-oxapent-1-yl]phenol was prepared by described methods[1].

Reference spectra for compounds 3, 7, 19 and 20 were available from a contract research institution in Cologne.

2,6-Di-*tert*-butyl-4-[5-(3-pyridyl)-4-oxapent-1-ylidene]cyclohexa-2,5-dien-1-one (8). The solution of Mrz 3/124 (355mg, 1.00mmol) (2) in 20ml of benzene was added at +5-7°C to a degassed and stirred solution of $K_3[Fe(CN)_6]$ (13.2g) and NaOH (10.0g) in 50ml of water. Emulsion turned emerald green. Stirring was continued for 1.5-2h at +5-7°C until the green color disappeared. Benzene layer was separated, washed with water until pH=6-7, dried and evaporated *in vacuo* at $t_{max.}$<30°C. Yield: 330mg (0.93 mmol, 93%) of bright yellow oil. Quinone methide 8 obtained was satisfactory pure for further preparations. ^1H NMR spectrum ($CDCl_3$, TMS),δ: 1.27 (18H, bs, 2*t*-Bu); 2.82 (2H, dt, J_1=7.4Hz, J_2=5.8Hz, $CH\underline{CH_2}$); 3.67 (2H, t, J=5.8Hz, CH_2O); 4.55 (2H, s, CH_2Py); 6.31 (1H, t, J=7.4Hz, CH); 6.86 and 7.26 (1H&1H, d, J=2.2Hz, C_6H_2); 7.27-7.37

(1H, m, Py-5H); 7.58-7.78 (1H, m, Py-4H); 8.49-8.64ppm (2H, m, Py-2H and Py-6H).

2,6-Di-*tert*-butyl-4-[5-(3-pyridyl)-4-oxapent-1-yl]-4-hydroxy-cyclohexa-2,5-dien-1-one (9).

A. Dry Mrz3/124 (2) (1.00g 2.8mmol) was added to a solution of benzyl-trimethylammonium tribromide (1.17g, 3.0mmol) in 10ml of methylene chloride. Water (10ml) was added under stirring in one portion to a reaction mixture. Stirring was continued for 6h. Reaction mixture was neutralized with saturated $NaHCO_3$. Organic layer was separated, washed with water, dried and filtered through 3g of *Kieselgel 60* to remove colored compounds. Filtrate was evaporated to give 0.82g (2.2mmol, 79%) of 9 as a pale yellow oil, crystallizing on standing. Crude quinol was crystallized from hexane.

B. Dry Mrz3/124 (2) (5.0g 14.1mmol) was added to a solution of methyl-3-(1,1,1-trimethylhydrazinium)-propionate tribromide (5.7g, 14.2mmol) in 60ml of methylene chloride. Water (60ml) was added under stirring in one portion to a reaction mixture. Stirring was continued for 5h. Reaction mixture was neutralized with saturated $NaHCO_3$. Organic layer was separated, washed with water, dried and filtered through 30g of *Kieselgel 60* to remove colored compounds. Filtrate was evaporated to give 3.9g (10.5mmol, 75%) of 9 as a yellow oil, crystallizing on standing. Crude quinol 9 was crystallized from hexane to obtain 2.3g (6.2mmol) of pure product 9.

^1H NMR spectrum ($CDCl_3$, TMS),δ: 1.18 (18H, bs, 2*t*-Bu); 1.22-1.98 (4H, m, CCH_2CH_2); 3.02 (1H, s, OH); 3.44 (2H, t, J=6.2Hz, CH_2O); 4.47 (2H, s,

CH_2Py); 6.47 (2H, s, C_6H_2); 7.22 (1H, m, Py-5H); 7.62 (1H, m, Py-4H): 8.44ppm (2H, m, Py-2H and Py-6H).

1-(3,5-Di-*tert*-butyl-4-hydroxyphenyl)-5-(3-pyridyl)-4-oxa-pentan-1-ol (10):

A solution of ketone 11 (2.12g, 5.7mmol) in 15ml of THF was added to $LiAlH_4$ (220mg, 5.80mmol) in 50ml of THF at -20°C during 20min. The reaction mixture was quenched with MeOH and usual workup gave 1.56g (4.2mmol, 74%) of crystalline substance. Crude alcohol 10 was crystallized twice from CCl_4 to give 1.30g of pure product 10. ^1H NMR spectrum ($CDCl_3$, TMS),δ: 1.42 (18H, s, 2*t*-Bu); 1.84-2.22 (2H, m, $CHCH_2$); 2.67 (1H, bs, CHOH); 3.68 (2H, m, CH_2O); 4.51 (2H, s, CH_2Py); 4.77 (1H, dd, J_1=4.8Hz, J_2=7.6Hz, ArCH); 5.16 (1H, s, ArOH); 7.11 (2H, s, C_6H_2); 7.22 (1H, m, Py-5H); 7.67 (1H, m, Py-4H); 8.47ppm (2H, m, Py-2H and Py-6H).

Reduction of ketone 11 with $NaBH_4$.

Ketone 11 (100mg, 0.27mmol) was dissolved in 2ml of i-propyl alcohol and $NaBH_4$ (12mg, 0.32mmol) was added. Reaction mixture was stirred at 60°C for 30min and poured into 8ml of ice-water. Resulting suspension was acidified to pH=4, and crystalline precipitate filtered off. Yield: 84mg (0.24mmol, 88%) of Mrz 3/124 (2). Melting point: 101-103°C.

3,5-Di-*tert*-butyl-4-hydroxy-ω-(3-pyridyl)oxymethylpropiophenone (11).

A. Dry Mrz3/124 (308mg, 0.87mmol) was added to a solution of benzyltrimethylammonium bromide (559mg, 1.40mmol) in 1.5ml of CH_2Cl_2. Water (1.5m) and *t*-BuOH (1.5ml) were added under stirring. The orange color disappears within 1h, reaction mixture was stirred for additional 2h and neutralized with saturated $NaHCO_3$. Organic layer was separated, washed with water, dried

and evaporated to give 487mg of dark-brown oil. Trituration with hexane affords crude crystalline ketone 11. Crystallization from ethyl acetate with addition of silica gel gives pure propiophenone 11. Yield: 240mg (0.65mmol, 75%).

B. Dry Mrz3/124 (9.1g, 25.6mmol) was added to a solution of methyl-3-(1,1,1-trimethylhydrazinium)-propionate tribromide (20.7g, 51.6mmol) in 150ml of CH_2Cl_2. Water (150ml) and *t*-BuOH (150ml) were added under stirring. The orange color disappears within 2h, reaction mixture was stirred for additional 1h and neutralized with saturated $NaHCO_3$. Organic layer was separated, washed with water, dried and evaporated to give 15.2g of dark-brown semi-crystalline solid. Trituration with hexane afforded crude crystalline ketone 11. Crystallization from ethyl acetate with addition of silica gel and activated charcoal gave pure propiophenone 11. Yield: 8.0g (21.7mmol, 85%).

^1H NMR spectrum ($CDCl_3$, TMS),δ: 1.44 (18H, s, 2*t*-Bu); 3.22 (2H, t, J=6.2Hz, $COCH_2$); 3.94 (2H, t, J=6.2Hz, CH_2O); 4.57 (2H, s, CH_2Py); 5.07 (1H, s, OH); 7.11-7.22 (1H, m, Py-5H); 7.53-7.91 (1H, m, Py-4H); 7.80(2H, s, C_6H_2); 8.33-8.62ppm (2H, m, Py-2H and Py-6H).

S-[1-(3,5-Di-*tert*-butyl-4-hydroxyphenyl)-5-(3-pyridyl)-4-oxapent-1-yl]glutathione (14).

A solution of quinone methide 8 (3.53g, 10.0 mmol) in 25ml of CH_2Cl_2 was added to a stirred mixture of glutathione (1.00g, 3.25 mmol) and tetrabutylammonium sulfate (1.10g, 3.25 mmol) in 25ml of water. The reaction mixture was vigorously stirred for 5 days and evaporated to give 7.0g of oily residue. Treatment with 150ml of anion exchanger *Toyopearl DEAE* in OH^- form and

elution with 1.5% NH_4OH afforded 4.0g of crystalline product, which was finally purified by flash chromatography on a 25x2.5cm *Kieselgel 60* column ($EtOH/NH_4OH$ 97/3) to give 680mg (1.03 mmol, 32%) of adduct 14 after crystallization from $Et_2O/CHCl_3$ (1/1). Melting point: 95-96°C.

^1H NMR spectrum (D_2O, DSS),δ: 1.37 (18H, s, 2*t*-Bu); 2.06-2.24 (4H, m, b-CH_2Glu and CH$\underline{CH_2}$); 2.40-2.56 (2H, m, γ-CH_2Glu); 2.56-2.96 (2H, m, CH_2S); 3.19 (2H, m); 3.37-3.59 (2H, m); 3.59-3.83 (3H, m, CHS and $\underline{CH_2}$COOH); 4.00 (1H, m, α-CHGlu); 4.50(1H, m, α-CHCys); 4.39-4.57 (2H, m, CH_2Py); 7.13 (2H, s, C_6H_2); 7.50 (1H, m, PyH-4); 7.78 (1H, m, PyH-4); 8.46ppm (4H, m, 2PyH-2 and 2PyH-6).

S-[1-(3,5-Di-*tert*-butyl-4-hydroxyphenyl)-5-(3-pyridyl)-4-oxapent-1-yl]cysteine (15).

A solution of quinone methide 8 (1.00g, 2.8 mmol) in 25ml of CH_2Cl_2 was added to a stirred mixture of cysteine (3.00g, 24.0 mmol) and tetrabutylammonium sulfate (2.00g, 5.8 mmol) in 25ml of water. The reaction mixture was vigorously stirred for 5 days. Adduct 19 was collected by filtration, washed with 50ml of cold water and crystallized from boiling water to give 760mg (1.60 mmol, 57%) of pure amino acid 15.

^1H NMR spectrum (DMSO-d_6 + D_2O, TMS),δ: 1.36 (18H, s, 2*t*-Bu); 2.07 (2H, m, ArCH$\underline{CH_2}$); 2.36-3.00 (4H, m, CH_2O, $\underline{CH_2}$CHCO); 3.16-3.62 (2H, m, ArCH, CHCO); 4.42 (2H, s, CH_2Py); 7.04 (2H, bs, C_6H_2); 7.38 (1H, m, Py-5H); 7.62 (1H, m, Py-4H); 8.51ppm (2H, m, Py-2H and Py-6H).

1-(3,5-Di-*tert*-butyl-4-hydroxyphenyl)-5-(3-pyridyl)-4-oxa-pentane N-oxide (16):

A solution of Mrz3/124 (2) (2.0g, 5.6mmol) in 12ml of CH_2Cl_2 was added at -10°C to a dried solution of 55% MCPBA (2.0g, 6.3mmol) in 10ml of CH_2Cl_2. The reaction mixture was warmed up to room temperature and stirred for 2h. The solution was washed with H_2O, sat. $NaHCO_3$, dried and evaporated to give 1.7g (4.6mmol, 82%) of N-oxide 16. ^1H NMR spectrum ($CDCl_3$, TMS),δ: 1.42 (18H, s, 2*t*-Bu); 1.69-2.18 (2H, m, $ArCH_2CH_2$); 2.62 (2H, m, $ArCH_2$); 3.53 (2H, t, 6.0Hz); 4.47 (2H, s, CH_2Py); 5.07 (1H, s, ArOH); 6.96 (2H, s, C_6H_2); 7.20 and 7.96-8.27ppm (4H, m, Py-H).

3[5-(3,5-Di-*tert*-butyl-4-hydroxyphenyl)-2-oxapentyl]pyrid-2-one (17) and 5[5-(3,5-di-*tert*-butyl-4-hydroxyphenyl)-2-oxapentyl]pyrid-2-one (18).

A solution of N-oxide 16 (1.5g, 4.0 mmol) in CH_2Cl_2 was cooled to -70°C and trifluoroacetic anhydride (650ml, 4.6 mmol) was added. The reaction mixture was stirred at -70°C for 10 min and triethylamine (700ml) was added. The solution was allowed to warm up to room temperature within 4h, washed with 2x20ml of saturated $NaHCO_3$, 3x20ml of water, dried and evaporated to give 1.63g of pale yellow oil. The isomers were separated on a 20x4.5 cm *Kieselgel 60* column (hexane/EtOAc 1/5) and crystallized from hexane/EtOAc (1:1). Yield: 3[5-(3,5-di-*tert*-butyl-4-hydroxyphenyl)-2-oxapentyl]pyrid-2-one (17) - 158mg (0.42 mmol, 11%) and 5[5-(3,5-di-*tert*-butyl-4-hydroxyphenyl)-2-oxapentyl]pyrid-2-one (18) - 220mg (0.59mmol, 15%).

^1H NMR spectrum of 17 ($CDCl_3$, TMS),δ: 1.39 (18H, s, 2*t*-Bu); 1.75-2.14 (2H, m, $ArCH_2CH_2$); 2.64 (2H, m, $ArCH_2$); 3.58 (2H, t, J=6.5Hz, CH_2O); 4.44 (2H, s, CH_2Py); 4.94 (1H, bs, ArOH); 6.25 (1H, t, J=6.3Hz, Py-5H); 6.94 (2H, s,

C_6H_2); 7.26 and 7.50 (2x1H, dd, J_1=6.3Hz, J_2=1.8Hz, Py-4H and Py-6H); 12.81ppm (1H, bs, PyOH).

^{1}H NMR spectrum of 18 ($CDCl_3$, TMS),δ: 1.42 (18H, s, 2*t*-Bu); 1.62-2.20 (2H, m, $ArCH_2\underline{CH_2}$); 2.57 (2H, m, $ArCH_2$); 3.47 (2H, t, J=7.3Hz, CH_2O); 4.24 (2H, s, CH_2Py); 5.13 (1H, bs, ArOH); 6.56 (1H, d, J=9.0Hz, Py-3H); 6.96 (2H, s, C_6H_2); 7.29 (1H, d, J=2.3Hz, Py-6H); 7.49ppm (1H, dd, J_1=9.0Hz, J_2=2.3Hz, Py-4H).

1-(3,5-Di-*tert*-butyl-4-hydroxyphenyl)-5-(3-pyridyl)-4-oxa-pent-1-ene (19):

A solution of quinone methide 8 (1.00g, 2.8 mmol) in 10ml of hexane/ethyl acetate mixture (10/4) was passed through a 20x2.4 cm *Kieselgel 60* column and eluted with hexane/ethylacetate (10/4). Product containing fractions (UV 254nm detection) were collected and evaporated to dryness to give colorless crystalline substance. Yield: 0.91g (2.6mmol, 92%). ^{1}H NMR spectrum ($CDCl_3$, TMS),δ: 1.43 (18H, s, 2*t*-Bu); 4.20 (2H, dd, J_1=5.9Hz, J_2=1.0Hz, CH_2O); 4.57 (2H, s, CH_2Py); 5.16 (1H, s, OH); 6.16 (1H, dt, J_1=5.9Hz, J_2=15.8Hz, $\underline{CH}CH_2$); 6.58 (1H, d, J=15.8Hz, ArCH); 7.20 (2H, s, C_6H_2); 7.29 (1H, m, Py-5H); 7.67 (1H, m, Py-4H); 8.42-8.64ppm (2H, m, Py-2H and Py-6H).

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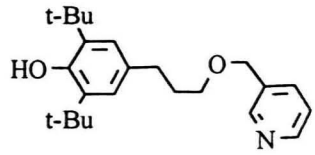

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Figure 1. Mrz 3/124 (*Nicanartine*)

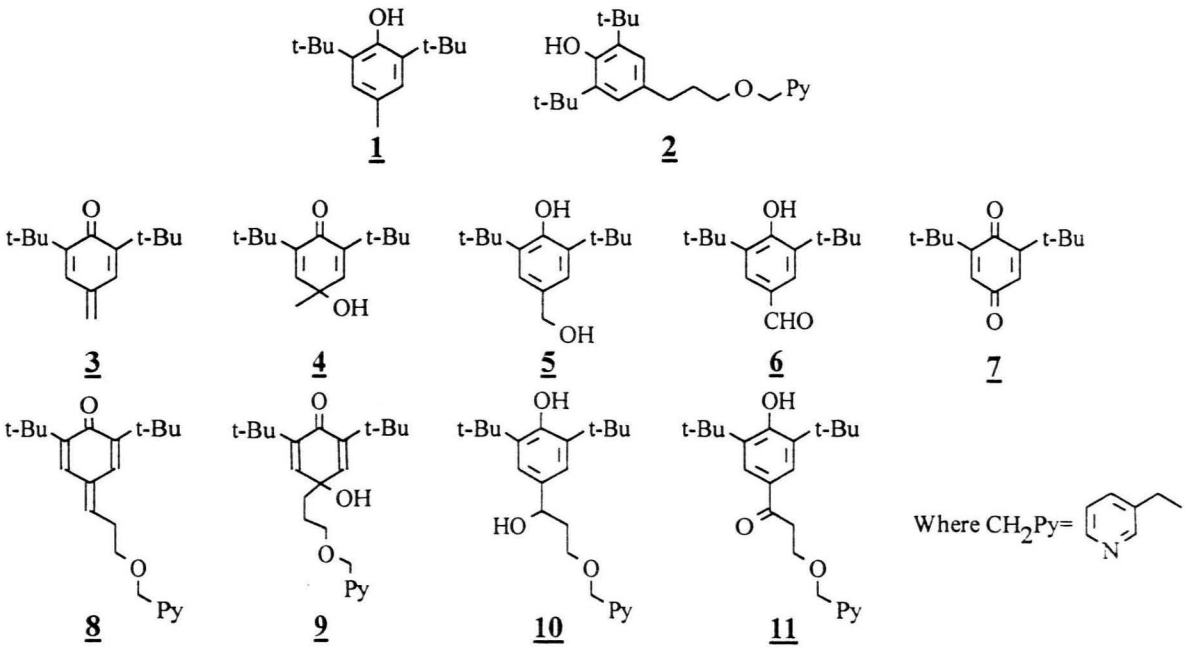

Figure 2. BHT oxidation products and corresponding Mrz 3/124 analogues

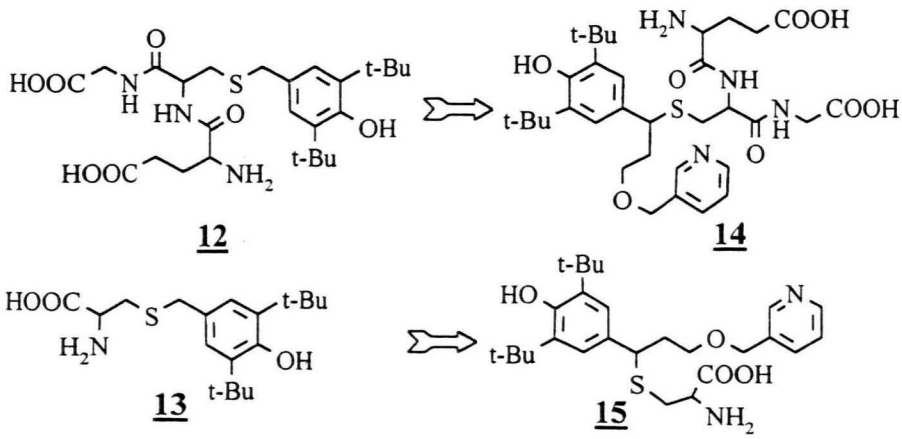

Figure 3. Adducts of BHT and Mrz 3/124 to glutathione and cysteine

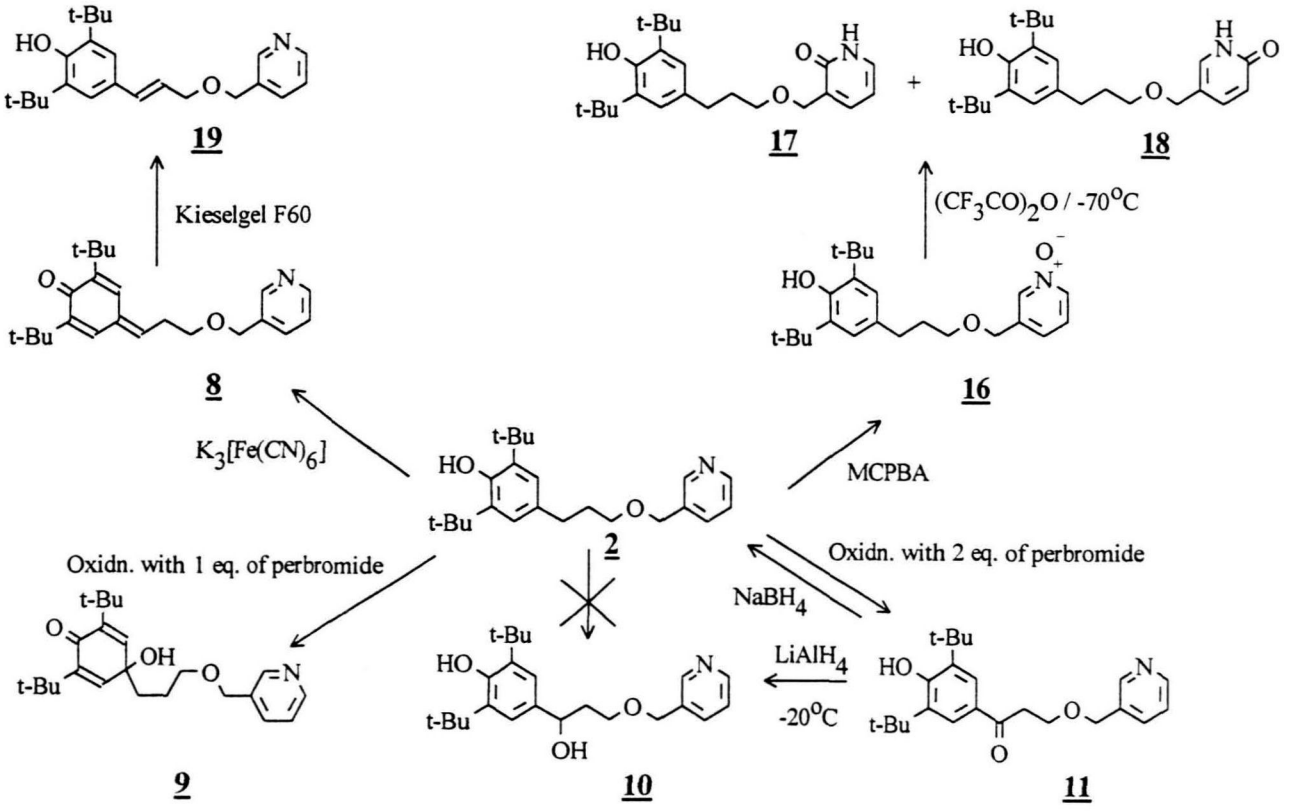

Figure 4. Oxidation of Mrz 3/124.

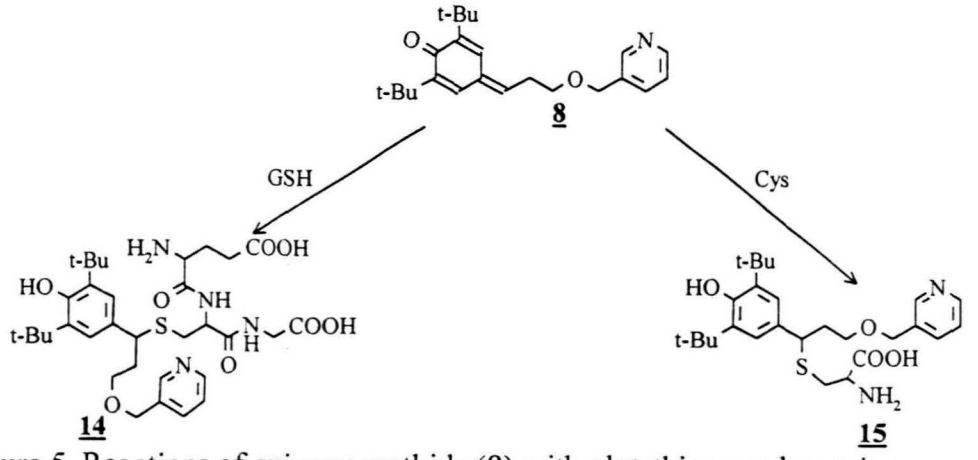

Figure 5. Reactions of quinone methide (8) with glutathione and cysteine

3. Pielikums

Proofs to Prof. Ivars Kalvinsh

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LATVIA

Oxidative Rearrangement of 3,5-di-*tert*-Butyl-4-hydroxy-benzaldehyde Acetals.

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3,5-di-*tert*-Butyl-4-hydroxy-benzaldehyde acetals undergo a rearrangement when oxidized with potassium ferricyanide in alkaline medium.

During the course of our studies on pharmacologically active 3,5-di-*tert*-butyl-4-hydroxybenzyl ethers we started to investigate the oxidation of both symmetrical and unsymmetrical 3,5-di-*tert*-butyl-4-hydroxy-benzaldehyde acetals in order to generate reactive quinone methides.

Here we wish to report a molecular rearrangement of 3,5-di-*tert*-butyl-4-hydroxy-benzaldehyde acetals **1** by treatment with potassium ferricyanide in benzene/aqueous sodium hydroxide. Common oxidation procedure[1] gave a mixture of products depending on the structure of the acetal **1** (see Scheme 1 and Table 1):

Insert Scheme1 here!

Insert Table 1 here!

We suggest the formation of quinone methide **6** to be the initial step. Addition of water and subsequent elimination of alcohol[2,3] leads to the formation of the ester **5** (see Scheme 2):

Insert Scheme2 here!

Meanwhile quinone methide **6** can undergo whether Claisen ester enolate[4] like rearrangement or radical reactions to give rearranged products **2**, **3**, and **4**. Sterical effects of *tert*-butyl groups facilitate the appearance of cyclohexadienone **2** type derivatives, while products **3** and **4** are less present in reaction mixture[5].

Experimental.

All ^{1}H NMR spectra were recorded on a 90 MHz Bruker WH-90 instrument. Acetals **1**(a-c) were prepared from 2,6-di-*tert*-butyl-4-[3-(3-pyridyl)-2-oxaprop-1-yl] phenol[6] and 3,5-di-*tert*-butyl-4-hydroxybenzaldehyde correspondingly according to known methods[3]. All oxidations were carried out in an argon atmosphere, solutions were degassed and saturated with argon prior to use. Column chromatography was performed on *Kieselgel* 60 using hexane / ethylacetate.

General procedure for the oxidation of 3,5-di-*tert*-butyl-4-hydroxybenzaldehyde acetals.

Acetal **1** (0.50 mmol) was dissolved in benzene (5ml) and under vigorous stirring added in one portion to a precooled (+6°C) solution of potassium ferricyanide (500mg, 1.52mmol) and sodium hydroxide (300mg, 7.5mmol) in 5ml of water. The reaction mixture turned green, stirring was continued for 30-60 min until the green color faded and TLC on *Kieselgel 60* showed disappearance of starting material.

Organic layer was separated, aqueous phase washed with 2x3ml of benzene and extracts washed with brine until neutral pH. The solution was dried over sodium sulfate, evaporated *in vacuo*, and products were separated on an 1.5x15cm column.

Methyl 3,5-di-*tert*-butyl-4-hydroxybenzoate (**5c**) 1.47(18H, s, t-Bu); 3.88(3H, s, OCH_3); 5.66(1H, s, OH); 7.89ppm(2H, s, C_6H_2).

3-Pyridyl-methyl 3,5-di-*tert*-butyl-4-hydroxybenzoate (**5a**) 1.47(18H, s, t-Bu); 5.36 (2H, s, CH_2Py); 5.69(1H, s, OH); 7.18-7.40(1H, m, Py-5H); 7.62-7.82(1H, m, Py-4H); 7.88(2H, s, C_6H_2); 8.56(1H, m, Py-6H); 8.71ppm(1H, m, Py-2H).

3-Pyridyl-methyl 3,5-di-*tert*-butyl-4-(3-pyridyl-methoxy)benzoate (**4a**) 1.28(9H, s, t-Bu); 1.41(9H, s, t-Bu); 4.88(2H, s, OCH_2Py); 5.38(2H, s, $COOCH_2Py$); 7.11-7.44(2H, m, Py-5H); 7.62-7.87(2H, m, Py-4H); 7.99(2H, s, C_6H_2); 8.49-8.78ppm(4H, m, Py-2H and Py-6H).

2,6-Di-*tert*-butyl-4-(3-pyridylmethyl)oxycarbonyl-4-(3-pyridylmethyl)-cyclohexa-2,5-dien-1-one **(2a)** 1.11(18H, s, t-Bu); 3.18(2H, s, CH_2Py); 5.19(2H, s, $COOCH_2Py$); 6.67(2H, s, C_6H_2); 6.89-7.69(4H, m, Py-5H and Py-4H); 8.18-8.64ppm(4H, m, Py-2H and Py-6H).

2,6-Di-*tert*-butyl-4-methyloxycarbonyl-4-(3-pyridylmethyl)-cyclohexa-2,5-dien-1-one **(2b)** 1.16(18H, s, t-Bu); 3.21(2H, s, CH_2Py); 3.79(3H, s, OCH_3); 6.74(2H, s, C_6H_2); 6.96-7.56(2H, m, Py-5H and Py-4H); 8.27(1H, d, J=1.5Hz, Py-2H); 8.41ppm(1H, dd., J_1=5.0Hz, J_2=1.5Hz, Py-6H).

2,6-Di-*tert*-butyl-4-(3-pyridylmethyl)oxycarbonyl-6-(3-pyridylmethyl)-cyclohexa-2,4-dien-1-one **(3a)** 0.98(9H, s, t-Bu); 1.09(9H, s, t-Bu); 2.71(1H, d, J=12.0Hz, CHPy); 3.53(1H, d, J=12.0Hz, CHPy); 5.04-5.38 (2H, m, $COOCH_2Py$); 7.78-7.76 (6H, m., 2xPy-4H, 2xPy-5H, 2xC=CH); 8.07-8.67ppm (4H, m., 2xPy-2H, 2xPy-6H).

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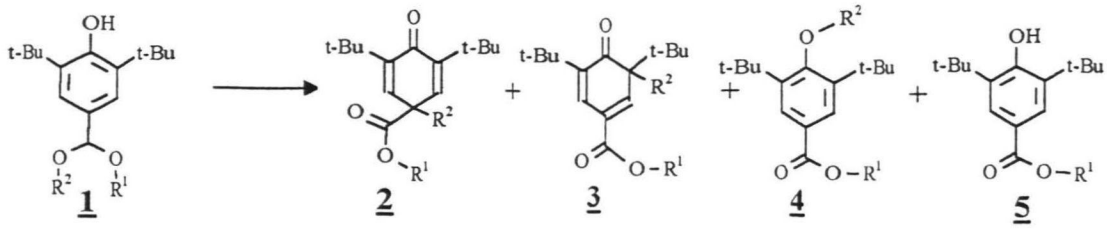
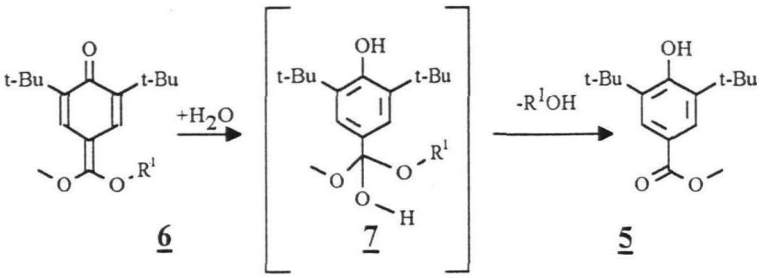


where: a) $R^1=R^2=CH_2(3\text{-Py})$

b) $R^1=Me$, $R^2=CH_2(3\text{-Py})$

c) $R^1=R^2=Me$

Scheme 1. Oxidation of 3,5-di-tert-butyl-4-hydroxybenzylacetals.



Scheme 2. The hydrolysis of quinone methide **6**.

Table 1. Yields of reaction products after oxidation of 3,5-di-*tert*-butyl-4-hydroxy-benzaldehyde acetals.

R^1	R^2	Yields*, (%)			
		2	3	4	5
CH_2(3-Py)	CH_2(3-Py)	76	2	3	10
Me	CH_2(3-Py)	90	-	-	-
Me	Me	-	-	-	91

**-Yields refer to isolated products.*

4. Pielikums

THE SYNTHESIS OF (3,5-DI-*TERT*-BUTYL-4-HYDROXYPHENYL)METHYL-(3-PYRIDYLALKYL)-ETHERS *VIA* 1-(3,5-DI-*TERT*-BUTYL-4-HYDROXYPHENYL)METHYL PYRIDINIUM SALTS.

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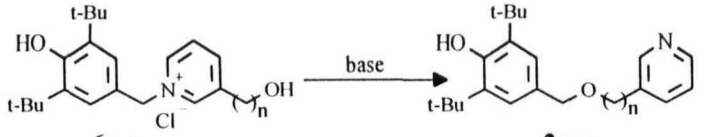
A convenient synthetic procedure for the preparation of (3,5-di-*tert*-butyl-4-hydroxyphenyl)methyl-(3-pyridyl-alkyl)-ethers **2a-c** from corresponding pyridinium salts **6a-c**

Where a) n=1
b) n=3
c) n=6



6a-c **2a-c**

THE SYNTHESIS OF (3,5-DI-*TERT*-BUTYL-4-HYDROXYPHENYL)METHYL-(3-PYRIDYLALKYL)-ETHERS *VIA* 1-(3,5-DI-*TERT*-BUTYL-4-HYDROXYPHENYL)METHYL PYRIDINIUM SALTS.

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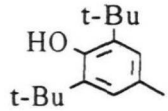
Abstract. The rearrangement of 1-(3,5-di-*tert*-butyl-4-hydroxyphenyl)methyl pyridinium salts under basic conditions is described. A method for the synthesis of (3,5-di-*tert*-butyl-4-hydroxyphenyl)methyl-(3-pyridylalkyl)-ethers is elaborated.

Since 1954 the 2,6-di-*tert*-butyl-4-methylphenol (**1**) (BHT, butylated hydroxy-toluene) has been one of the most widely used preservatives added to food, cosmetics and drugs. Combination of the BHT residue with a nicotinic acid precursor - pyridyl-3-carbinol[1] gave (3,5-di-*tert*-butyl-4-hydroxyphenyl)methyl-(3-pyridyl)-methyl ether (**2**) with high hypolipidemic and antiatherosclerotic activity.



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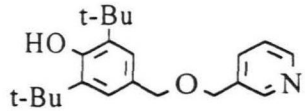

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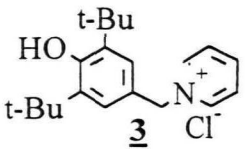
* to whom correspondence should be addressed.

However, the known synthesis of **2** and its analogues[1,2] is a complicated 7 stage procedure including protection and deprotection steps with the total yield of 25%. Introduction of unprotected (3,5-di-*tert*-butyl-4-hydroxyphenyl)methyl moiety into a molecule may cause problems due to rapid oxidation and formation of complex quinoid systems. Only a few methods allow direct benzylation with (3,5-di-*tert*-butyl-4-hydroxyphenyl)methyl halides[3]. The usage of tertiary amines[4] or quaternary acylammonium salts[5] afford better results. Recently we offered the 1-(3,5-di-*tert*-butyl-4-hydroxyphenyl)methyl pyridinium chloride (**3**) as a synthetic tool for the synthesis of (3,5-di-*tert*-butyl-4-hydroxyphenyl)methylmalonic esters[6].



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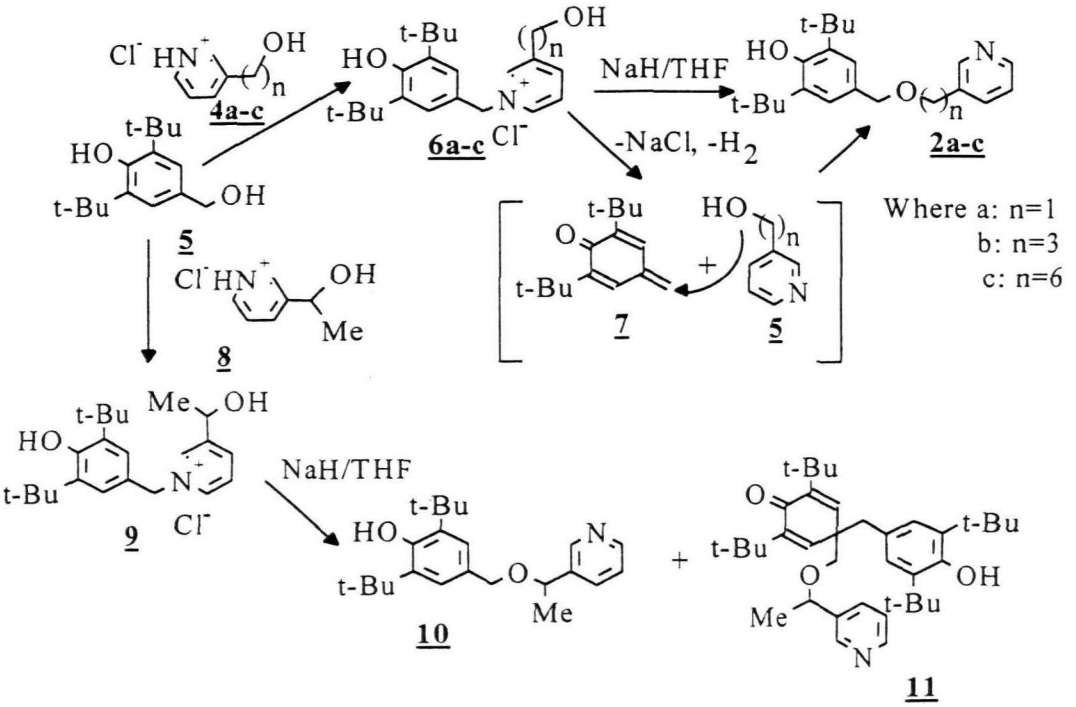
Here we wish to report a convenient synthetic procedure for the preparation of (3,5-di-*tert*-butyl-4-hydroxyphenyl)methyl-(3-pyridylalkyl)-ethers *via* 1-(3,5-di-*tert*-butyl-4-hydroxyphenyl)methyl pyridinium salts.

Interaction of pyridylalcohol hydrochlorides **4a-c** with benzylalcohol **5** results in corresponding pyridinium salts **6a-c**. Further rearrangement of **6a-c** under basic conditions gives ethers **2a-c** (scheme 1). As a first step we suggest initial formation of quinone methide **7** followed by the nucleophilic attack of the pyridylalcohol. Yields depend on the base, temperature and solvent applied (table 1). Sodium hydride in THF at -15°C appears to be the optimum conditions for this rearrangement. More drastic reaction conditions cause (see table 1) dimerization

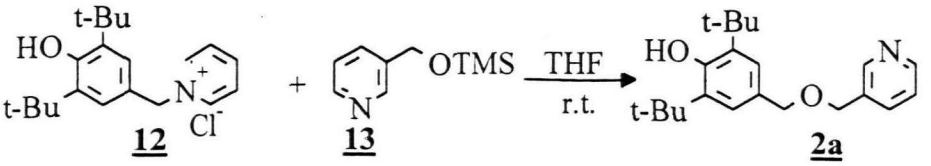
of quinone methide **7** resulting in formation of large amount of colored products[7]. Chain length of pyridylalcohols **4a-c** has no significant effect on the reaction yields (see table 2). Lower yields referred for **2b** and **2c** could be explained by certain loss of substance after crystallization from hexane. In the contrary secondary alcohol **8** gives a mixture of two main products: desired ether **10** and cyclohexadienone derivative **11** (see table 3). Appearance of adduct **11** could be explained as the dimerization of intermediate quinone methide **7** followed by addition of an alcohol **8** molecule. Obviously more sterically hindered alcohol **8** reacts slower with the quinone methide **7** and dimerization of the latter becomes favorable.

Scheme1. Synthesis of di-*tert*-butyl-benzylethers.



Usage of solid sodium hydride in THF is a serious drawback of the method described. The reaction needs certain activation by temperature increase and then, after the process is started, the temperature in reaction medium should be kept at -15°C to get good yield of product. If the exothermic process is not suppressed, the yields decrease dramatically (see table 1). Attempts to use soluble bases, to avoid activation, failed (table 1, runs 6 and 7). Finally we tried to prepare ether **2a** according to the so called "silyl method"[8]. Treatment of pyridinium salt **12** with O-trimethylsilylpyridylcarbinol **13** in THF at room temperature gives the target ether **2a** in 82% yield (scheme 2):

Scheme 2. Preparation of ether **2a** from O-trimethylsilylpyridylcarbinol.



In conclusion, the procedure presented here is useful for the preparation of the title compounds because of high yields, simple synthetic procedure and low reagent costs.

Experimental. All ^{1}H NMR spectra were recorded on a 90 MHz Bruker WH-90 instrument. Melting points were determined on a Boetius table and are uncorrected. All reactions were carried out in an argon atmosphere. Solvents were prepared before use as described in literature[9].

General procedure for preparation of pyridinium salts 4a-c, 9 and 12.

1-(3-Pyridyl)ethanol hydrochloride (**8**) (4.75g 29.7mmol) was dissolved in 50ml acetonitrile and benzylalcohol (**5**) (20g, 84.6mmol) was added. Reaction mixture was refluxed for 5h, evaporated to dryness, oily residue triturated with ether, filtered and washed with ether to give 8.63g (87%) of **9** as a pale yellow crystalline substance.

General procedure for rearrangement of pyridinium salts 4a-c and 9.

Sodium hydride (412mg, 17.17mmol) was suspended in 120ml THF. The mixture was cooled to -35°C and dry pyridinium salt **6a** (5.0g, 13.76mmol) was added in one portion. Reaction mixture was warmed up to -10°C and kept until the reaction begins (hydrogen evolution). The mixture was stirred at -15°C for 3h, then allowed to warm up to room temperature and poured into 150ml ice cold brine. Organic layer was separated, washed twice with 50ml brine. Aqueous phase was extracted twice with 50ml ether, organic extracts were dried over sodium sulfate and evaporated to give 4.18g of yellow crystals. Recrystallization from hexane gave 3.87g (11.83mmol, 86%) of ether **2a**.

2,6-Di-*tert*-butyl-4-[(3,5-di-*tert*-butyl-4-hydroxyphenyl)methyl]-4-[1-(pyridyl-3)-ethoxy-methyl]-cyclohexa-2,5-dien-1-one (11).

Pyridinium salt **9** (1.026g, 3.10mmol) was treated with 105.0 mg (3.5mmol) 80% NaH in 30ml THF according to the procedure described above. Usual workup gave 1.036g of brown oil. Trituration with hexane afforded 472.2 mg

(0.84 mmol) of **11**. Residual oil was chromatographed on 50g *Kieselgel 60* in hexane/EtOAc (5/2) to give 57.5mg (0.10 mmol) of **11** and 260.1mg (0.76mmol, 24%) of ether **10.** Total yield of cyclohexadienone **11**: 529.7mg (0.95mmol, 61%). Melting point: 145-6°C

^{1}H NMR spectra ($CDCl_3$, TMS) δ: 1.16 and 1.19(18H, s, t-Bu); 1.31(3H, d, J=5.8Hz, CCH_3); 1.40(18H, s, t-Bu); 2.72(1H, d, J=12.6Hz, CHAr); 2.95(1H, d, J=12.6Hz, CHAr); 3.12(1H, d, J=8.8Hz, CHO); 3.26(1H, d, J=8.8Hz, CHO); 4.34(1H, q, J=5.8Hz, OCHPy); 5.00(1H, s, OH); 6.45(1H, d, J=2.4Hz, C=CH); 6.60(1H, d, J=2.4Hz, C=CH); 6.86(2H, s, C_6H_2); 7.10-7.30(1H, m, Py-5H); 7.50-7.70(1H, m, Py-4H); 8.49ppm(2H, m, Py-2H and Py-6H).

Reaction of O-trimethylsilylpyridylmethanol with pyridinium salt 12.

O-Trimethylsilylpyridylmethanol (**13**) (2.0ml, 10.6mmol) was dissolved in 5ml THF and added to a suspension of 3.3g (10.0mmol) pyridinium salt **12** in 10ml THF. Reaction mixture was stirred at room temperature for 24h and poured into 30ml of ice-cold brine. Usual workup gave 2.69g (8.22mmol, 82%) of ether **2a**.

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Table 1. Preparation of (3,5-di-*tert*-butyl-4-hydroxyphenyl)methyl-(3-pyridyl)methyl ether (**2a**).

No. of run	Base / Solvent	Time / t, (°C)	Yield (%)
1.	NaH / THF	3h / -15	94
2.	NaH / THF	2h / r.t.	45
3.	BaO / MeCN	3h / reflux	41
4.	K_2CO_3 / MeCN	5h / reflux	40
5.	K_2CO_3 / DMF	1h / reflux	traces[a)]
6.	Et_3SiH / THF	24h / r.t	no rxn.
7.	DABCO / THF	24h / r.t.	0[b)]
8.	NaOMe /MeCN	2h / reflux	traces[a)]

[a)]- a lot of colored reaction products were obtained, trace amount of **2** was obtained after flash chromatography.
[b)]- pyridyl carbinol as a free base and alkylated DABCO were detected in reaction mixture.

Table 2. ^{1}H NMR spectra, melting points and yields of (3,5-di-*tert*-butyl-4-hydroxyphenyl)methyl-(3-pyridylalkyl)-ethers ($CDCl_3$, TMS).

No. of cmpd.	δ, (ppm)						Yield, (%)	M.P., (°C)
	t-Bu	CH_2Ar	$Py(CH_2)_nO$	ArOH	C_6H_2	Py		
2a	1.42	4.53	4.44 (2H, s)	5.18	7.11	7.22(1H, m); 7.69(1H, m); 8.48(2H, m).	94	109-10
2b	1.42	4.37	1.75-2.05 (2H, m); 2.72 (2H, t, J=7.5Hz); 3.47 (2H, t, J=6.0Hz)	5.18	7.10	7.15(1H, m); 7.43(1H, m); 8.40(2H, m).	67	72-3
2c	1.41	4.37	1.0-1.8 (8H, m); 2.59 (2H, t, J=7.5Hz); 3.44 (2H, t, J=6.5Hz).	5.15	7.10	7.06(1H, m); 7.44(1H, m); 8.40(2H, m).	72	61-2
10	1.43	4.34	1.53 (3H, d, J=6.2Hz); 4.55 (1H, q, J=6.2Hz).	5.24	7.11	7.22-7.38(1H, m); 7.62-7.82(1H, m); 8.51-8.67(2H, m).	24[a]	93-4

[a] -61% of adduct **11** was isolated.

Table 3. ^{1}H NMR spectra, melting points and yields of 1-(3,5-di-*tert*-butyl-4-hydroxyphenyl)methyl pyridinium salts ($CDCl_3$, TMS).

No.of cmpd.	R	δ, ppm						Yield, (%)	M.P., (°C)
		t-Bu	R	ArOH	CH_2N^+	C_6H_2	Py		
6a*	CH_2OH	1.36	4.67 (2H, s)	7.20	5.71	7.29	8.07 (1H, m); 8.47(1H, m); 9.09-9.15(2H,m)	82	216-9, dec.
6b	$(CH_2)_3OH$	1.41	2.00 (2H, m); 3.06 (2H, t, J=7.0Hz); 3.64 (2H, t, J=6.0Hz)	5.41	6.00	7.29	7.74 (1H, m); 8.19 (1H, m); 8.64 (1H, m); 9.75 (1H, bs).	73	210-2, dec.
6c	$(CH_2)_6OH$	1.38	0.8-1.75 (8H, m); 2.83 (2H, m); 3.46 (2H, m).	5.44	6.02	7.29	7.98(1H, m); 8.21(1H, m); 9.05-9.55(2H,m);	80	
9	CH_3CHOH	1.41	1.53(3H, d, J=6.0Hz); 5.13(1H, q, J=6.0Hz); 6.3(1H, b.s.)	5.44	5.89	7.29	7.91 (1H, m); 8.44 (1H, m); 8.45 (1H, m); 9.51 (1H, m).	84	200-2, dec
12*	H	1.37	-	7.24	5.74	7.31	8.15 (1H, m); 8.58(2H, m); 9.21 (2H, m).	81	238-42, dec