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Dehydroabietylamine derived bistetrazoles from ultrasound-assisted pseudo-seven-component Ugi reactions act as efficient and selective inhibitors of cholinesterases

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ABSTRACT

The reaction of dehydroabiethylamine (**DHA**) with isocyanides and formaldehyde produces varying products depending on the conditions employed. In a 4-component Ugi reaction using benzyl isocyanide, paraformaldehyde, and TMS-azide, the anticipated tetrazole is formed. Nevertheless, other isocyanides were unsuccessful in these conditions. Upon substituting paraformaldehyde with formalin and utilizing ultrasound instead, bis-tetrazole formation was obtained in a pseudo-7-component reaction. A bis-benzyl-substituted tetrazole **5** demonstrated significant AChE and BChE inhibition in Ellman's assays. Molecular modeling corroborated these results, with compound **5** identified as a mixed-type inhibitor for both enzymes.

1. Introduction

Tetrazoles were first accessed by Thiele [1], Bladin [2,3] and Lossen [4] about 140 years ago, and since then this class of compounds has found wide applications in various fields [5]. 1*H*-tetrazoles as well as 1, 5-disubstituted tetrazoles are bioisosteres of carboxylic acids and cis-amides, respectively, and have a wide range of biological activities, such as antiplatelet, diuretic, analgesic, phosphordiesterase inhibitory activity; they are also used as antihypertensive drugs thereby acting as angiotensin II type 1 receptor antagonists [6]. The bioisosteric nature also explains their comparatively high stability combined with mostly low toxicity [7].

Although innumerable tetrazoles have been described in the literature, the number of tetrazole-substituted di- and triterpenes has remained small over all these years [8–11]. For example, Petrova et al., 2021 described the synthesis, cytotoxicity, and α-glucosidase inhibitory activity of tetrazole-substituted triterpenes and of sitosterols, respectively [12]. A tetrazole derived from 20,29-dihydrobetulin (holding also an opened A-ring) was of low cytotoxicity [13], and for a limited number of tetrazole-substituted ursolic acid derivatives some SAR studies concerning their anticancer activity were performed [14]. Some of these derivatives were also found to be inhibitors of hypoxia inducible factor 1α (HIF-1α) [15] or held some activity against *Toxoplasma gondii* [16].

Although many derivatives of dehydroabietylamine (**DHA**, Scheme 1) have been described [17], the potential of tetrazole-substituted derivatives has not yet been investigated. Of particular interest to us seemed to study their ability to inhibit cholinesterases (*ee*AChE and *b*BChE) [18]. The inhibition of cholinesterases, especially AChE and BChE, still plays an important role in improving the quality of life of people with neurodegenerative diseases, especially Alzheimer's disease but also Parkinson's disease [19,20].

2. Results and discussion

Expanding our previous studies of multicomponent reactions (MCRs) [21,22], the Ugi azide 4-CR appeared to be a suitable method for the synthesis of some model compounds (Scheme 1).

While the reaction of **DHA** with benzyl isocyanide, TMS-azide and paraformaldehyde gave 49 % of the expected tetrazole **1**, analogous reactions with alkyl isocyanides did not give the corresponding products either at room temperature or under reflux. Ultrasound-assisted reaction [23] of **DHA** with *n*-butyl isocyanide or *tert*. butyl isocyanide, cyclohexyl isocyanide or benzyl isocyanide, with TMS-azide and formalin gave the doubly substituted products **2**–**5** by a pseudo-7-CR-Ugi reaction [24].

To our knowledge, the formation of such bis-tetrazoles on a terpenoid backbone has never been reported before. However, only a few

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reactions to substituted *N, N*-bis-(1-benzyl-1*H*)-tetrazol-5-yl-cyclopropanamines [25] starting from primary amines have also been described. A few bis-tetrazoles had been described earlier starting from cyclic ketimines and reacting them with substituted hydrazines in a 4-component reaction [26].

A possible mechanism (Scheme 2) for this pseudo-7-CR Ugi reaction [24] would proceed via an initial reaction of formaldehyde with **DHA** to the corresponding iminium ion A.

Intermediate A would be attacked by the isocyanide used in excess leading to intermediate B, which would react with hydrazoic acid (resulting from the reaction of TMS-azide with solvent methanol) yielding intermediate C that would cyclize to the corresponding mono-tetrazole. The latter compound, would ultimately lead to the formation of the bis-tetrazoles by subsequent reaction with further isocyanide, hydrazoic acid, and formaldehyde. This formation of the bis-tetrazoles would be preferable to the formation of the mono-tetrazole, since the amino group of the mono-tetrazole-substituted **DHA** derivative is more nucleophilic and basic than is parent **DHA**. By using ultrasound, the reaction - in contrast to the non-ultrasound-assisted variant - is significantly accelerated, and the yields of products are significantly increased. The TMS-OMe formed during the reaction is hydrolyzed, and hexamethyl-disiloxane was formed (as detected by an *in-situ* ^{29}Si NMR of the reaction mixture, $\delta = 0.31$ ppm). Worthwhile to mention, that six new bonds are formed during this pseudo-7-CR Ugi reaction.

The structural elucidation of the obtained bis-tetrazoles was carried out by instrumental analytical methods, as will be shown by the example of **4**. The ESI-MS spectrum shows, among others, a quasi-molecule ion at $m/z = 614.2$ (100 %, $[M+H]^+$). The microanalysis additionally confirms a molecular formula of $C_{36}H_{55}N_9$.

The ^{1}H NMR and ^{13}C NMR spectra show the expected framework of the **DHA** skeleton, the signals for the cyclohexyl residues, and in the ^{13}C NMR spectra the signal typical for tetrazoles is found at $\delta = 151.0$ ppm.

Fig. 1 shows the ^{15}N NMR spectrum of **4** including an assignment of the individual signals; the signal positions agree very well with literature data of similar compounds as well as a spectrum simulation (MestReNova 14.3.3.).

Tetrazoles have only scarcely been tested for their inhibitory activity onto AChE or BChE. Thereby, their bioactivity was low. However, this holds not true for the compounds of this investigation. Ellman's assays were performed, and thereby the bisbenzyl derivative **5** showed excellent activity for AChE and BChE while alkyl substituted derivatives were a rather selective inhibitor for AChE but not for BChE. The results from these assays are compiled in Table 1 and depicted in Fig. 2.

Extra kinetic measurements showed **5** acting as a mixed type inhibitor for both enzymes with $K_i = 1.17$ μM and $K_i' = 2.73$ μM (for AChE) and $K_i = 0.99$ μM and $K_i' = 0.98$ μM (for BChE), respectively. Thus, this bis-tetrazole is about as good as an inhibitor as galantamine – a first line symptomatic treatment of moderate dementia. Dixon and Cornish-Bowden plots for dibenzyl substituted **5** are depicted in Fig. 3.

A basic requirement for future and further biological testing is that the compounds show no or only low cytotoxicity. Initial SRB assays using the malignant cell lines A375, HT29, MCF-7, A2780, HeLa and non-malignant murine fibroblasts NIH 3T3 as well as human embryonic kidney cells (HEK293) showed that for all compounds $EC_{50} > 30$ μmol - this is generally considered non-cytotoxic.

To better understand these results, appropriate molecular modeling calculations were performed.

For the molecular modeling the crystal structure of *ee*AChE from *Torpedo californica* (3I6M) and for BChE the structure of *h*BChE (4BDS) were used.

For AChE, the scores confirm - more or less-the experimentally determined inhibition percentages; only between **1** and **5** no clear difference could be found from these calculations.

The respective crystal structures were prepared using the QuickPrep function of MOE followed by applying Protonote3D. For docking, program GOLD was applied. The calculated GOLDScores are compiled in Table 2.

Inspection of the calculations shows for **3** that the **DHA** backbone is localized inside the binding pocket. The tetrazoles interact with Arg289 and Tyr334 via hydrogen bonds. The latter also holds an H-bond to a hydrogen to the **DHA**.

The tetrazol moieties of compound **5** are located inside the binding pocket. One of the tetrazole rings holds a H-bond with Gly118. The preferred location and orientation of **5** are depicted in Fig. 4.

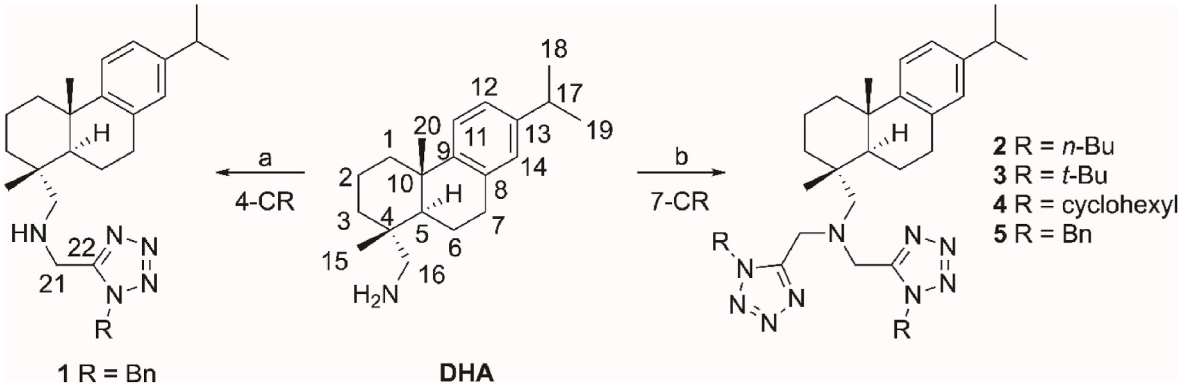
Similarly, Fig. 5 summarizes the results from the calculations for BChE and compound **5**.

In summary, the molecular modeling calculations revealed that in the case of AChE, the **DHA** derivatives holding aromatic residues bind better than those with (cyclo)-alkyl residues. This could be due to pi-pi interactions. Docking of **4** gave the worst results, which is also consistent with the experimentally determined inhibition percentages.

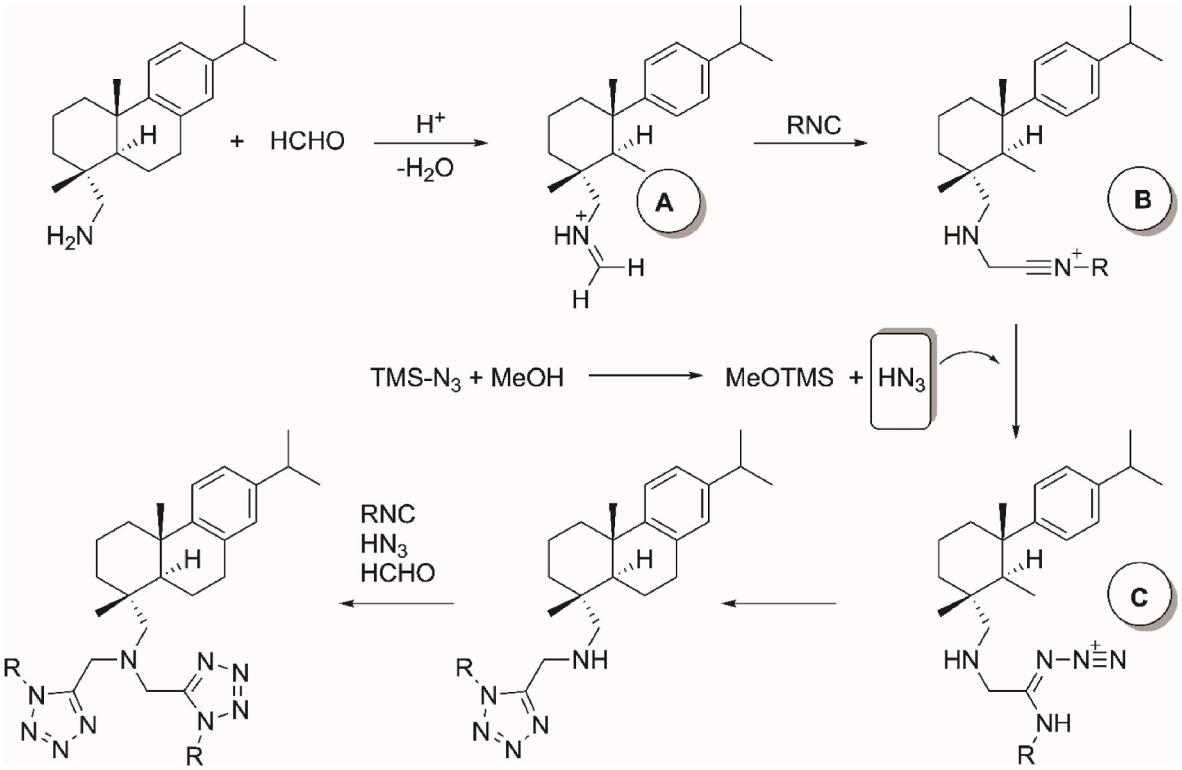
In the case of BChE, the results correlate also very well with the experimental results from the inhibition studies. The GOLDScore of **5** is significantly better than the scores calculated for the other compounds. Most of the interactions occurred in BChE with Trp82 (Trp84 in AChE) and Tyr332 (for BChE; equivalent to Tyr334 in AChE). The significantly larger binding pocket of BChE has a positive effect on the binding of **DHA**.

3. Conclusion

The products of the reaction between dehydroabiethylamine (**DHA**) and isocyanides, and formaldehyde differ depending on the reaction conditions. When benzyl isocyanide, paraformaldehyde and TMS-azide were used in a 4-CR Ugi reaction, the expected tetrazole was formed. However, the utilization of other isocyanides in these reaction



Scheme 1. Formation of tetrazolyl-substituted **DHA** derivative **1** by a 4-CR Ugi reaction and of bis-tetrazolyl-substituted **DHA** derivatives **2**–**5** by pseudo-7CR reactions: a) MeOH, R–NC, TMS-azide, paraformaldehyde, 20 °C, 24 h, 49 %; b) R–NC, TMS-azide, formalin, ultrasound: → **2** (56 %), **3** (54 %), **4** (72 %), **5** (58 %).



Scheme 2. Putative mechanism of the pseudo-7-CR-Ugi reaction leading to the formation of bis-tetrazolyl-substituted **DHA** derivatives.

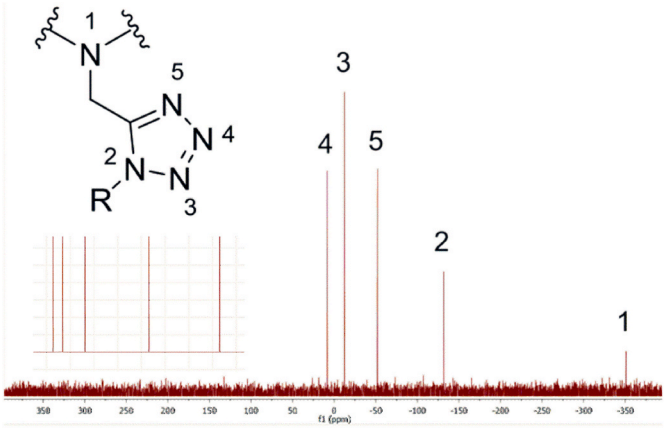


Fig. 1. ^{15}N NMR spectrum of **4**; the small insert shows a calculated spectrum for this compound (MestReNova 14.3.3.).

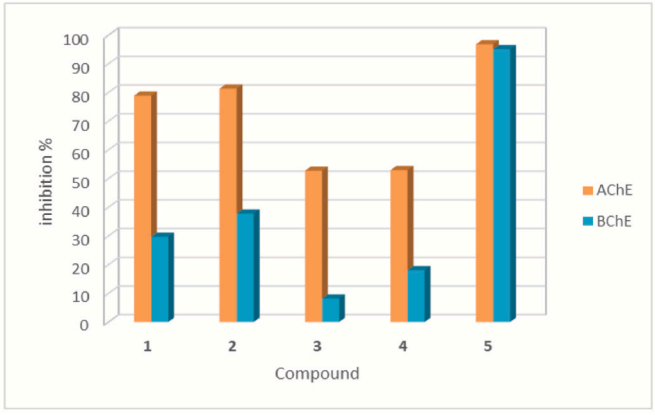


Fig. 2. Inhibition of AChE and BChE by compounds **1**–**5**.

Table 1
Percentage of inhibition of compounds **1**–**5** (concentration: 10 μM) in Ellman's assays employing *ee*AChE and *b*BChE; galantamine was used as a positive standard.

Compound	% inhibition AChE	% inhibition BChE
1	78.9	29.7
2	81.3	37.8
3	52.7	8.2
4	52.9	18.1
5	96.8	95.1
Galantamine	88.9	44.3

conditions led to failure. Using formalin instead of paraformaldehyde and ultrasound in the reaction resulted in the formation of bis-tetrazoles in a pseudo-7CR reaction. A bis-benzyl-substituted tetrazole (**5**) demonstrated strong inhibition of both AChE and BChE in Ellman's assays. The corresponding mono-substituted tetrazole **1**, however, held only moderate inhibition for AChE and minimal inhibitory activity for BChE. The most effective inhibitor (i.e., **5**) acted as a mixed-type inhibitor for both enzymes. Accompanying molecular modeling calculations satisfactorily explicated these findings.

4. Experimental

4.1. General

Equipment and calculation methods are described in the Supplementary Materials file (also depicting the NMR spectra of **1**–**5**). In addition, the results from the calculation for compounds **1**–**5** are presented in higher resolution.

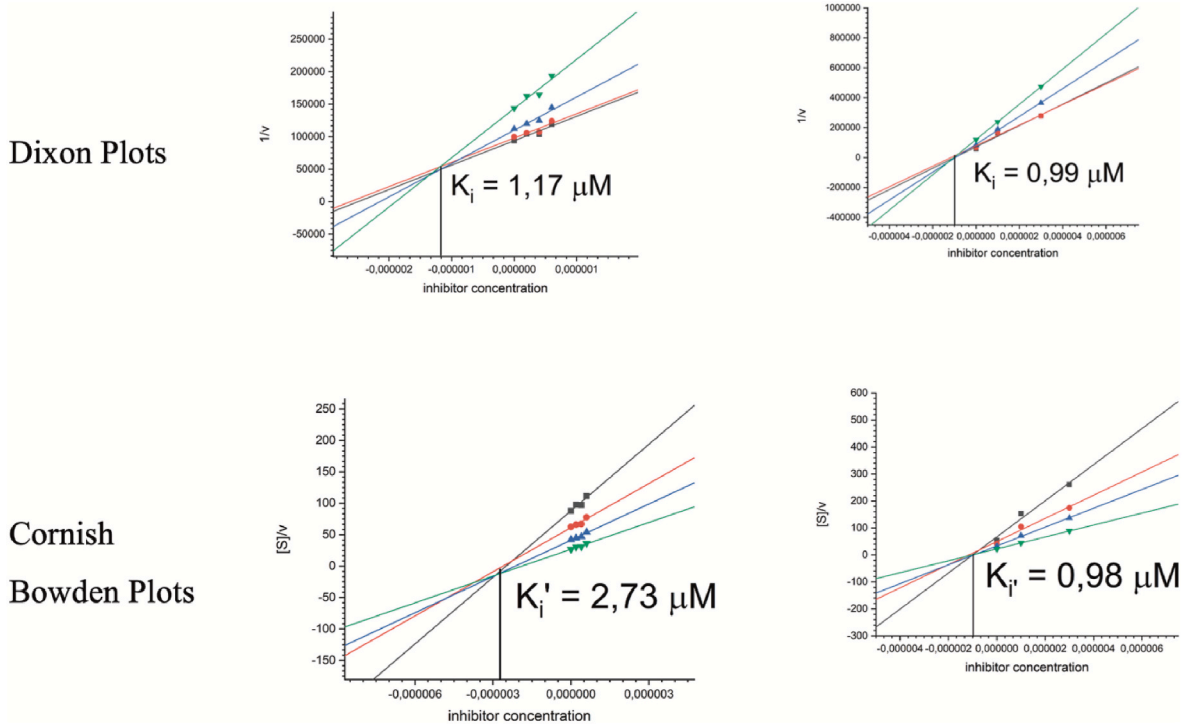


Fig. 3. Dixon and Cornish-Bowden plots for **5** and enzymes AChE (left) and BChE (right).

Table 2
Calculated GoldScores for compounds **1**–**5** and enzymes AChE and BChE.

Compound	AChE	BChE
1	−56.32	−52.12
2	−41.34	−56.11
3	−43.74	−40.29
4	−33.01	−41.60
5	−51.18	−70.8

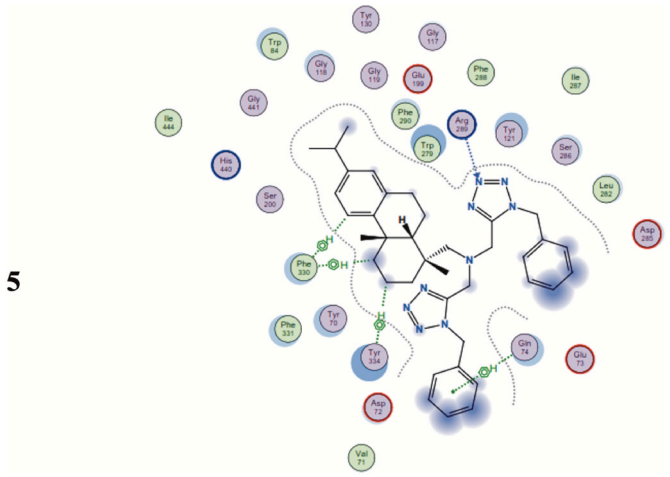


Fig. 4. Calculated preferred localization and orientation of **5** and AChE.

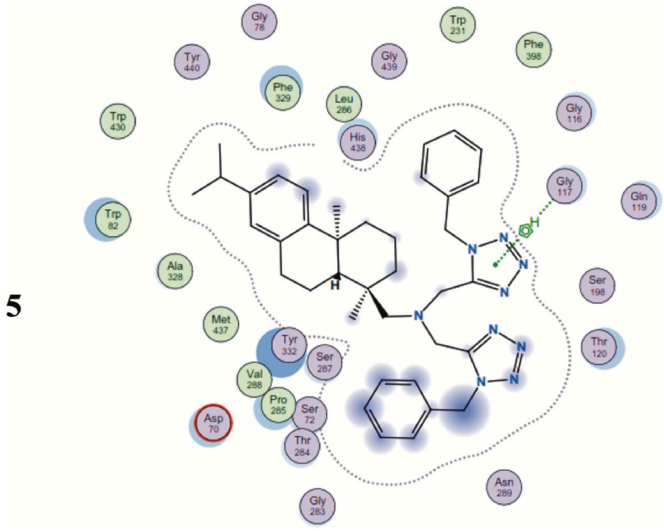


Fig. 5. Calculated preferred localization and orientation of **5** and BChE.

4.2. Syntheses

4.2.1. N-[(1-Benzyl-1H-tetrazol-5-yl)methyl]abieta-8,11,13-trien-18-amine (1)

DHA (500 mg) was dissolved in methanol (20 mL), benzylisocyanide (0.22 mL, 1.8 mmol), TMS-azide (0.24 mL, 1.8 mml) and paraformaldehyde (200 mg) were added, and the reaction was stirred at ambient temperature for 5 h. The volatiles were removed under reduced pressure, and the residue subjected to chromatography (silica gel, hexanes/ethyl acetate 10 % → 30 %) to afford **1** (390 mg, 49 %) as a colorless solid; m.p. 68–70 °C; $R_f = 0.39$ (silica gel, hexanes/ethyl acetate, 7:3); $[\alpha]_D^{20} = +26.4°$ (*c* 0.216, $CHCl_3$); UV–Vis (MeOH): λ_{max} (log ε) = 268 nm (3.89), 276 nm (3.84); IR: $\nu = 2926$ *m*, 1497 *m*, 1456*s*, 1382*w*, 1361*w*, 1240*w*, 1109 *m*, 1073*w*, 1030*w*, 989*w*, 882*w*, 822 *m*, 751*s*, 723*vs*, 667*w*, 630*w*, 580*w*, 458*w* cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ = 7.33–7.27 (*m*, 3H, 26-H, 27-H, 28-H), 7.22–7.17 (*m*, 2H, 25-H, 29-H), 7.15 (*d*, $J = 8.2$ Hz, 1H, 11-H), 6.98 (*d*, $J = 8.1$ Hz, 1H, 12-H), 6.87 (*s*, 1H, 14-H), 5.70 (*s*, 2H, 23-H), 4.07–3.92 (*m*, 2H, 21-H), 2.96–2.72 (*m*, 3H, 17-H, 7-H), 2.57 (*d*, $J = 11.8$ Hz, 1H, 16-H_a), 2.40–2.18 (*m*, 2H, 16-H_b, 1-H_a), 1.87–1.58 (*m*, 4H, 6-H, 2-H), 1.49 (*dd*, $J = 11.8$, 2.8 Hz, 1H, 5-

H), 1.44–1.28 (*m*, 3H, 1-H_b, 3-H), 1.23 (*s*, 3H, 18-H), 1.21 (*s*, 3H, 19-H), 1.19 (*s*, 3H, 20-H), 0.91 (*s*, 3H, 15-H) ppm; ^{13}C NMR (101 MHz, $CDCl_3$): δ = 152.9 (C-22), 147.3 (C-9), 145.8 (C-13), 134.6 (C-8), 133.7 (C-24), 129.3 (C-26, C-28), 129.0 (C-27), 127.8 (C-25, 29), 126.9 (C-14), 124.3 (C-11), 124.0 (C-12), 61.2 (C-16), 51.2 (C-23), 45.6 (C-5), 42.9 (C-21), 38.5 (C-1), 37.5 (C-10), 37.2 (C-4), 36.3 (C-3), 33.6 (C-17), 30.1 (C-7), 25.4 (C-20), 24.1 (C-18, C-19), 19.2 (C-15), 19.0 (C-2), 18.8 (C-6); MS (ESI, MeOH/$CHCl_3$, 4:1): *m/z* (%) = 480.3 (100 %, $[M+Na]^+$), 458 (12 %, $[M+H]^+$); analysis calcd for $C_{29}H_{39}N_5$ (457.67): C 76.11, H 8.59, N 15.30; found C 75.89, H 8.78, N 15.17.

4.2.2. N,N-Bis[(1-butyl-1H-butyl-1H-tetrazol-5-yl)methyl]abieta-8,11,13-trien-18-amine (2)

DHA (500 mg) was dissolved in methanol (20 mL), *n*-butyl isocyanide (0.38 mL, 3.6 mmol), TMS-azide (0.48 mL, 3.6 mmol) and formalin (37 %, 0.4 mL, 5.2 mmol) were added, and the reaction was stirred at rt for 5 h by immersion of the flask in an ultrasonic cleaning bath (60 W). Removal of the volatiles followed by chromatography (silica gel, hexanes/ethyl acetate 10 % → 30 %) gave **2** (560 mg, 56 %) as a colorless solid; m.p. 53–55 °C; R_f = 0.74 (silica gel, hexanes/ethyl acetate, 1:1); $[\alpha]_D^{20}$ = +13.5° (*c* 0.200, $CHCl_3$); UV–Vis (MeOH): λ_{max} (log ε) = 268 nm (3.81), 276 nm (3.81); IR: ν = 2958*s*, 2932*s*, 2871 *m*, 2214 *m*, 1675*w*, 1498 *m*, 1459*s*, 1380 *m*, 1234*w*, 1104*s*, 1057 *m*, 974 *m*, 822 *m*, 754*s*, 631*w* cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.13 (*d*, *J* = 8.2 Hz, 1H, 11-H), 6.98 (*dd*, *J* = 8.2, 2.0 Hz, 1H, 12-H), 6.86 (*d*, *J* = 2.0 Hz, 1H, 14-H), 4.25 (*t*, *J* = 7.3 Hz, 4H, 21-H), 4.18–3.96 (*m*, 4H, 23-H), 2.99–2.70 (*m*, 5H, 16-H, 17-H, 7-H), 2.29 (*td*, *J* = 12.3, 3.2 Hz, 1H, 1-H_a), 1.82–1.55 (*m*, 8H, 6-H, 2-H, 24-H), 1.52–1.45 (*m*, 1H, 5-H), 1.43–1.22 (*m*, 7H, 1-H_b, 3-H, 25-H), 1.22 (*s*, 3H, 18-H), 1.20 (*s*, 3H, 19-H), 1.18 (*s*, 3H, 20-H), 0.90 (*t*, *J* = 7.3 Hz, 6H, 26-H), 0.80 (*s*, 3H, 15-H) ppm; ^{13}C NMR (101 MHz, $CDCl_3$): δ = 151.9 (C-22), 147.3 (C-9), 145.9 (C-13), 134.2 (C-8), 126.9 (C-14), 124.1 (C-11), 124.0 (C-12), 67.1 (C-16), 48.6 (C-23), 47.4 (C- 21), 45.3 (C-5), 39.4 (C-10), 38.5 (C-1), 37.6 (C-4), 36.8 (C-3), 33.6 (C-17), 31.7 (C-24), 30.0 (C-7), 25.6 (C-20), 24.1 (C-18), 24.1 (C-19), 19.7 (C-25), 19.2 (C-6), 18.7 (C-2), 18.4 (C-15), 13.5 (C-26) ppm; MS (ESI, MeOH): *m/z* (%) = 585.4 (100 %, $[M+Na]^+$), 562 (18 %, $[M+H]^+$); analysis calcd for $C_{32}H_{51}N_9$ (561.82): C 68.41, H 9.15, N 22.44; found C 68.19, H 9.41, N 22.15.

4.2.3. N,N-Bis[(1-tert-butyl-1H-tetrazol-5-yl)methyl]abieta-8,11,13-trien-18-amine (3)

Following the procedure for the synthesis of **2**, from **DHA** (500 mg, 1.75 mmol), *tert*-butyl isocyanide (0.4 mL, 3.5 mmol), TMS-azide (0.48 mL, 3.6 mmol) and formalin (0.4 mL, 5.2 mmol) followed by chromatography (silica gel, hexanes/ethyl acetate 10 % → 30 %) **3** (540 mg, 54 %) was obtained as a colorless solid; m.p. 50–52 °C; R_f = 0.64 (silica gel, hexanes/ethyl acetate, 1:1); $[\alpha]_D^{20}$ = +4.0° (*c* 0.082, $CHCl_3$); UV–Vis (MeOH): λ_{max} (log ε) = 268 nm (3.75), 276 nm (3.72); IR: ν = 3207*w*, 2966 *m*, 2208*s*, 1461 *m*, 1396 *m*, 1372*s*, 1227*s*, 1105*s*, 1026 *m*, 974 *m*, 823 *m*, 755 *m*, 585 *m*, 440*w* cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.14 (d, J = 8.2 Hz, 1H, 11-H), 6.97 (dd, J = 7.9, 1.9 Hz, 1H, 12-H), 6.85 (d, J = 2.0 Hz, 1H, 14-H), 4.69–4.40 (m, 4H, 21-H), 3.05–2.70 (m, 5H, 16-H, 17-H, 7-H), 2.26 (d, J = 12.7 Hz, 1H, 1-H_a), 1.60 (s, 18H, 24-H, 25-H, 26-H), 1.58 (m, 6H, 2-H, 3-H_a, 5-H), 1.47–1.32 (m, 2H, 1-H_b, 3-H_b), 1.22 (s, 3H, 19-H), 1.21 (s, 3H, 18-H), 1.19 (s, 3H, 20-H), 0.93 (s, 3H, 15-H) ppm; ^{13}C NMR (101 MHz, $CDCl_3$): δ = 152.6 (C-22), 147.3 (C-9), 145.5 (C-13), 134.2 (C-8), 126.8 (C-14), 124.1 (C-11), 123.9 (C-12), 68.5 (C-16), 60.8 (C-23), 50.8 (C-21), 45.2 (C-5), 39.9 (C-10), 38.3 (C-1), 37.5 (C-4), 36.6 (C-3), 33.4 (C-17), 30.1 (C-7), 29.5 (C-24, C-25, C-26), 28.4, 25.7 (C-20), 24.0 (C-19), 19.2 (C-6), 18.6 (C-2), 18.2 (C-15) ppm; MS (ESI, MeOH/$CHCl_3$, 4:1): *m/z* (%) = 584.3 (100 %, $[M+Na]^+$), 594 (54 %, $[M + MeOH + H]^+$), 562 (40 %, $[M+H]^+$); analysis calcd for $C_{32}H_{51}N_9$ (561.82): C 68.41, H 9.15, N 22.44; found C 68.13, H 9.38, N 22.11.

4.2.4. N,N-Bis[(1-cyclohexyl-1H-tetrazol-5-yl)methyl]abieta-8,11,13-trien-18-amine (4)

Following the procedure for the synthesis of **2**, from **DHA** (500 mg, 1.75 mmol), cyclohexyl isocyanide (0.44 mL, 3.6 mmol), TMS-azide (0.48 mL, 3.6 mmol) and formalin (0.4 mL, 5.2 mmol) followed by chromatography (silica gel, hexanes/ethyl acetate 10 % → 30 %) **4** (520 mg, 48 %) was obtained as a colorless solid; m.p. 85–87 °C; R_f = 0.64 (silica gel, hexanes/ethyl acetate, 1:1); $[\alpha]_D^{20}$ = +12.3° (*c* 0.168, $CHCl_3$); UV–Vis (MeOH): λ_{max} (log ε) = 268 nm (3.81), 276 nm (3.81); IR: ν = 2932*s*, 2859 *m*, 1447*s*, 1380*w*, 1099*s*, 1008*w*, 974*w*, 894 *m*, 822 *m*, 753 s cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.14 (*d*, *J* = 8.2 Hz, 1H, 11-H), 6.97 (*dd*, *J* = 8.2, 2.0 Hz, 1H, 12-H), 6.86 (*d*, *J* = 2.0 Hz, 1H, 14-H), 4.21 (*hept*, *J* = 5.1 Hz, 2H, 23-H), 4.15–3.98 (*m*, 4H, 21-H), 3.00–2.68 (*m*, 5H, 16-H, 17-H, 7-H), 2.29 (*dt*, *J* = 12.8, 3.4 Hz, 1H, 1-H_a), 2.07–1.80 (*m*, 12H, 28-H, 27-H_a, 25-H_a, 24-H), 1.79–1.55 (*m*, 8H, 6-H, 2-H, 26-H), 1.49 (*dd*, *J* = 10.9, 3.7 Hz, 1H, 5-H), 1.41–1.23 (*m*, 7H, 1-H_b, 3-H, 27-H_b, 25-H_b), 1.22 (*s*, 3H, 18-H), 1.20 (*s*, 3H, 19-H), 1.19 (*s*, 3H, 20-H), 0.81 (*s*, 3H, 15-H) ppm; ^{13}C NMR (101 MHz, $CDCl_3$): δ = 151.0 (C-22), 147.2 (C-9), 145.9 (C-13), 134.2 (C-8), 127.0 (C-14), 124.1 (C-11), 124.1 (C-12), 67.1 (C-16), 58.0 (C-23), 48.6 (C-21), 45.4 (C-5), 39.3 (C-4), 38.5 (C-1), 37.7 (C-10), 36.9 (C-3), 33.6 (C-17), 33.1 (C-24, C-28), 32.9 (C-24, C-28), 30.2 (C-7), 25.8 (C-20), 25.2 (C-25, C-27), 25.1 (C-25, C-27), 24.9 (C-26), 24.1 (C-18), 24.1 (C-19), 19.3 (C-6), 18.7 (C-2), 18.6 (C-15) ppm; ^{15}N NMR (51 MHz, $CDCl_3$): δ = 8.41 (N-4), −12.36 (N-3), −52.15 (N-5), −131.86 (N-2), −350.89 (N-1) ppm; MS (ESI, MeOH/$CHCl_3$, 4:1): *m/z* (%) = 614.2 (100 %, $[M+H]^+$), 646 (96 %, $[M + MeOH + H]^+$), 636 (90 %, $[M+Na]^+$); analysis calcd for $C_{36}H_{55}N_9$ (613.90): C 70.43, H 9.03, N 20.53; found C 70.23, H 9.31, N 20.25.

4.2.5. N,N-Bis[(benzyl-1H-tetrazol-5-yl)methyl]abieta-8,11,13-trien-18-amine (5)

Following the procedure for the synthesis of **2**, from **DHA** (500 mg, 1.75 mmol), benzyl isocyanide (0.43 mL, 3.6 mmol), TMS-azide (0.48 mL, 3.6 mmol) and formalin (0.4 mL, 5.2 mmol) followed by chromatography (silica gel, hexanes/ethyl acetate 10 % → 30 %) **5** (330 mg, 58 %) was obtained as a colorless solid; m.p. 69–73 °C; R_f = 0.75 (silica gel, chloroform/MeOH, 95:5); $[\alpha]_D^{20}$ = +26.8° (*c* 0.177, $CHCl_3$); UV–Vis (MeOH): λ_{max} (log ε) = 218 nm (4.97), 276 nm (4.07); IR: ν = 2955 m, 2928 m, 2866w, 1497 m, 1455s, 1431 m, 1377w, 1360w, 1107 m, 1073w, 977w, 822 m, 749w, 723vs, 706 s cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$): δ = 7.31–7.28 (*m*, 6H, 26-H, 27-H, 28-H), 7.13–7.07 (*m*, 5H, 11-H, 25-H, 29-H), 6.97 (*dd*, *J* = 8.2, 2.0 Hz, 1H, 12-H), 6.82 (*d*, *J* = 2.0 Hz, 1H, 14-H), 5.64–5.56 (*m*, 4H, 23-H), 4.03 (*d*, *J* = 15.6 Hz, 1H, 21-H_a), 3.95 (*d*, *J* = 15.5 Hz, 2H, 21-H_b), 2.89–2.74 (*m*, 3H, 7-H_a, 16-H_b, 17-H), 2.69 (*d*, *J* = 14.7 Hz, 1H, 16-H_a), 2.55 (*ddd*, *J* = 17.9, 11.2, 7.9 Hz, 1H, 7-H_b), 2.24–2.18 (*m*, 1H, 1-H_a), 1.66–1.53 (*m*, 3H, *2*-H, 6-H_a), 1.49 (*dt*, *J* = 14.0, 3.6 Hz, 1H, 6-H_b), 1.42–1.37 (*m*, 1H, 3-H_a), 1.29 (*dd*, *J* = 12.2, 2.7 Hz, 1H, 5-H), 1.26–1.24 (*m*, 1H, 1-H_b), 1.22 (*d*, *J* = 6.9 Hz, 6H, 18, 19-H), 1.12 (*s*, 3H, 20-H), 0.95 (*td*, *J* = 12.9, 3.9 Hz, 1H, 3-H_b), 0.70 (*s*, 3H, 15-H) ppm; ^{13}C NMR (126 MHz, $CDCl_3$): δ = 151.7 (22-C), 147.2 (9-C), 145.8 (13-C), 134.3 (8-C), 133.3 (24-C), 129.4 (26-C, 28-C), 129.1 (27-C), 127.5 (25, 29-C), 126.9 (14-C), 124.1 (11-C), 124.1 (12-C), 67.4 (16-C), 51.1 (23-C), 48.8 (21-C), 45.8 (5-C), 39.2 (10-C), 38.3 (1-C), 37.5 (4-C), 36.7 (3-C), 33.6 (17-C), 29.9 (7-C), 25.6 (20-C), 24.1 (18-C), 24.1 (19-C), 19.2 (2-C), 18.6 (6-C), 17.8 (15-C) ppm; MS (ESI, MeOH/$CHCl_3$, 4:1): *m/z* (%) = 652.6 (100 %, $[M+Na]^+$), 630.6 (14 %, $[M+H]^+$); analysis calcd for $C_{38}H_{47}N_9$ (629.86): C 72.46, H 7.52, N 20.01; found C 72.21, H 7.88, N 19.73.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

No data was used for the research described in the article.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ejmcr.2023.100124.

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